Upsala Journal of Medical Sciences. 2013; 118: 16–22

informa
healthcare

ORIGINAL ARTICLE

Tumor response evaluation criteria for HCC (hepatocellular carcinoma) treated using TACE (transcatheter arterial chemoembolization): RECIST (response evaluation criteria in solid tumors) version 1.1 and mRECIST (modified RECIST): JIVROSG-0602

YOZO SATO[1], HIROKAZU WATANABE[2], MIYUKI SONE[2], HIROAKI ONAYA[2], NORIAKI SAKAMOTO[2], KEIGO OSUGA[3], MASAHIDE TAKAHASHI[2], YASUAKI ARAI[2] AND JAPAN INTERVENTIONAL RADIOLOGY IN ONCOLOGY STUDY GROUP (JIVROSG)

[1]*Department of Diagnostic and Interventional Radiology, Aichi Cancer Center Hospital, Nagoya, Japan,* [2]*Department of Diagnostic Radiology, National Cancer Center Hospital, Tokyo, Japan, and* [3]*Department of Diagnostic Radiology, Osaka University Graduate School of Medicine, Osaka, Japan*

Abstract

Background. Two standard sets of criteria are used to evaluate the tumor response of hepatocellular carcinoma (HCC): RECIST (Response Evaluation Criteria in Solid Tumors) and modified RECIST (mRECIST). The purpose was to compare two tumor response evaluation criteria, RECIST version 1.1 and mRECIST, for HCC treated using transcatheter arterial chemoembolization (TACE).

Methods. The radiological findings of patients who underwent TACE for HCCs in a multicenter clinical trial were examined. Sixty-five lesions in 21 patients treated with TACE without mixing iodized-oil were evaluated. The tumor size was evaluated by measuring the entire lesion, including the necrotic part, using RECIST version 1.1, whereas only the contrast-enhanced part observed during the arterial phase was measured using mRECIST. Five radiologists independently measured each lesion twice. To evaluate the inter-criteria reproducibility, the complete response (CR) rate, the response rate, the kappa statistics, and the proportion of agreement (PA) for response categories were calculated. The same analyses were conducted for inter- and intra-observer reproducibility.

Results. In the inter-criteria reproducibility study, the CR rate and the response rate obtained using mRECIST (56.9% and 79.7%) were higher than those obtained using RECIST version 1.1 (9.2% and 43.1%). In the inter- and intra-observer reproducibility study, mRECIST exhibited an 'almost perfect agreement', while RECIST version 1.1 exhibited a 'substantial agreement'.

Conclusions. Considerable differences in the CR rate and the response rate were observed. From the viewpoint of the high inter- and intra-observer reproducibility, mRECIST may be more suitable for tumor response criteria in clinical trials of TACE for HCC.

Key words: *Hepatocellular carcinoma, modified RECIST, RECIST version 1.1, reproducibility, tumor response*

Introduction

Two standard sets of criteria are used to evaluate the tumor response of hepatocellular carcinoma (HCC) treated using loco-regional therapy, such as transcatheter arterial embolization (TACE): RECIST (Response Evaluation Criteria in Solid Tumors) criteria (1) and modified RECIST (mRECIST) criteria (2).

RECIST criteria were published by the National Cancer Institute in 2000 with the objective of unifying

Correspondence: Hirokazu Watanabe, Department of Diagnostic Radiology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Fax: +81-3-3542-3815. E-mail: hirwatan@ncc.go.jp

(Received 8 June 2012; accepted 6 September 2012)

ISSN 0300-9734 print/ISSN 2000-1967 online
DOI: 10.3109/03009734.2012.729104

the criteria used for response assessments. These criteria evaluate the unidimensional measurement of the longest diameter of the tumor lesions and have been used in most oncology trials. However, a number of questions and issues have arisen, leading to the development of revised RECIST (version 1.1) criteria (3). In the RECIST version 1.1 criteria, the major changes included the number of lesions to be assessed, the assessment of pathological lymph nodes, confirmation of a response, disease progression, and the necrotic tumor size (i.e. in cases where a lesion which was solid at baseline has become necrotic in the center, the longest diameter of the entire lesion should be followed).

In 2000, a panel of experts on HCC from the European Association for the Study of the Liver (EASL) agreed that estimating the reduction in viable tumor volume (as recognized using enhanced spiral computed tomography (CT)) should be considered the optimal method for assessing the local response to treatment in patients with HCC (4). Since then, most authors reporting the results of loco-regional therapy for HCC have evaluated tumor response according to this recommendation (5,6).

The aforementioned expert panel continued the concept of viable tumor endorsed by EASL and adapted the unidimensional measurement as a substitute for the bidimensional one in the determination of tumor response for target lesions in HCC (7). These amendments confirmed the American Association for the Study of Liver Disease (AASLD)–Journal of the National Cancer Institute (JNCI) guidelines and were defined as 'modified RECIST (mRECIST)' criteria (2). Therefore, mRECIST criteria were developed for loco-regional therapies to HCC. On the other hand, RECIST version 1.1 criteria were developed for systemic therapies; however, RECIST version 1.1 criteria are used in many oncology trials including loco-regional therapies for the treatment of HCC.

A study investigating the inter-criteria reproducibility between the older versions of criteria (RECIST version 1.0 and EASL) has been reported (8). Furthermore, a comparative study of tumor response by the updated criteria (RECIST version 1.1 and mRECIST) has been published (9). However, to the best of our knowledge, the inter- and intra-observer reproducibility between RECIST version 1.1 and mRECIST has not been investigated or reported.

Using these standardized criteria for evaluating tumor response in clinical trials, reproducible results should be obtained by all investigators. For a surrogate marker such as tumor response for therapy, both 'precision' (observer consistency study) and 'accuracy' (validation study comparing to gold standard) are evaluated. From the viewpoint of 'precision', we compared RECIST version 1.1 and mRECIST criteria by evaluating the inter- and intra-observer reproducibility.

The purpose of the present study was to clarify the differences in tumor response as evaluated using two updated sets of criteria (RECIST version 1.1 and mRECIST) by assessing the inter-criteria reproducibility. Moreover, another purpose of the present study was to investigate which set of criteria was superior for use as tumor response evaluation criteria in clinical trials of TACE for HCC by assessing the inter- and intra-observer reproducibility.

Materials and methods

We analyzed the radiological findings of patients who underwent pan-hepatic TACE for multiple HCCs in a multicenter clinical trial. In this trial, the eligibility criteria included patients with untreated, bilobar multiple HCCs, compensated Child–Pugh A or B cirrhosis, and the absence of vascular invasion or extrahepatic spread. TACE was performed using cisplatin (IA call, Nihon-Kayaku; 35–65 mg/m^2) and gelatin particles without mixing iodized-oil. The present study was conducted in accordance with the Helsinki Declaration, and the protocols were approved by the institutional review board. Informed written consent for the treatment protocols, including the secondary use of treatment-associated documents, was obtained from each patient. Twenty-one patients were entered from 19 July 2005 to 15 May 2007.

Image analysis

All patients underwent a dynamic study performed using a multi-slice CT scanner with non-ionic contrast medium. CT scans were obtained within two weeks before TACE and one month after TACE. Tumor assessments were made using a 5-mm interval, and axial images were obtained during the unenhanced phase, the arterial phase, and the portal venous or equilibrium phase.

Tumor response evaluation

Response was defined according to RECIST version 1.1 criteria measuring the entire lesion, including the necrotic part. On the other hand, mRECIST were used to evaluate the lesion taking tumor necrosis, recognized by the non-enhanced areas, into account. Both guidelines adopted the unidimensional measurement (Figure 1).

According to RECIST version 1.1 criteria, a complete response (CR) was defined as the disappearance

of all target lesions; a partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter of the target lesions; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of the target lesions; and stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor a sufficient increase to qualify for PD.

According to mRECIST criteria, CR was defined as the absence of enhanced tumor areas during the arterial phase, reflecting complete tissue necrosis; PR was defined as at least a 30% decrease, PD was defined as at least a 20% increase in the sum of the longest diameter in the enhanced tumor areas; and SD was defined using the same definition as that used in RECIST version 1.1 criteria.

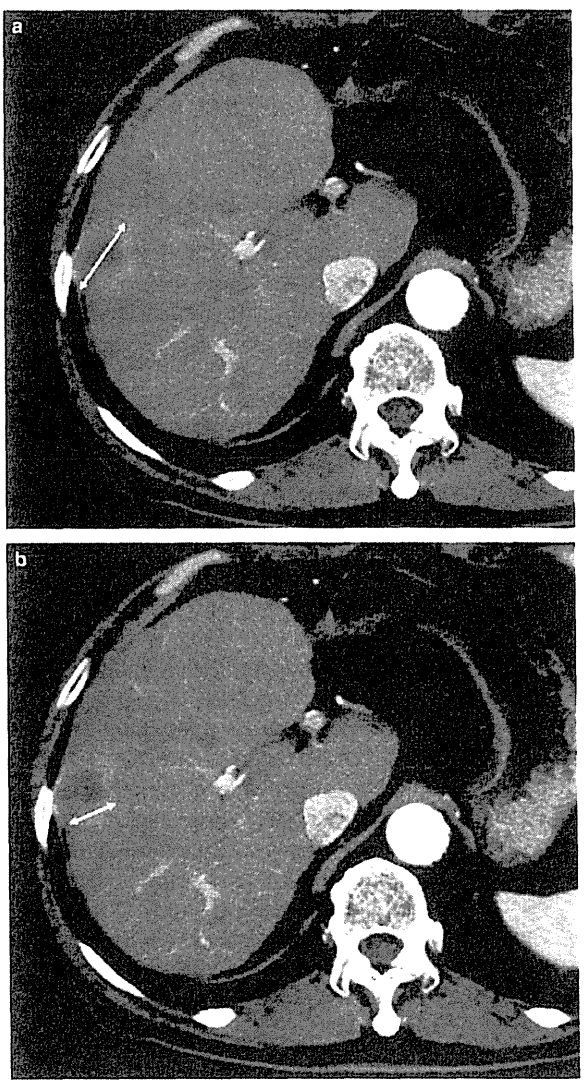

Figure 1. A: RECIST ver. 1.1: Response was defined according to a unidimensional measurement of the entire lesion, including the necrotic part. B: mRECIST: Response was defined according to a unidimensional measurement of the viable part, excluding the necrotic part.

Evaluation methods

Five observers measured 65 lesions in 21 patients independently. A total of 325 measurements were made for the first measurement. The second measurement was performed independently by the same five observers. The sum of the longest diameters for all the target lesions was calculated for baseline and post-treatment. The baseline sum was used as the reference from which the objective tumor response could be calculated. The percentage changes were calculated as the post-treatment value divided by the pre-treatment value. The percentage changes were then classified using RECIST version 1.1 and mRECIST tumor response classification systems. Tumor response was categorized as CR, PR, SD, or PD based on both sets of criteria. Furthermore, the CR rate and the response rate were also calculated.

All the images were collected from each institution and supplied to the Japan Interventional Radiology in Oncology Study Group (JIVROSG) Data Center using the WEB system.

Analysis of inter-criteria reproducibility

To examine the inter-criteria reproducibility between RECIST version 1.1 and mRECIST criteria, we estimated the kappa statistics and the proportion of agreement for the CR, PR, SD, and PD categories among the five observers. The data for the first measurements were analyzed to evaluate the inter-criteria reproducibility.

Analysis of inter-observer reproducibility

To examine the inter-observer reproducibility among the five observers, we estimated the kappa statistics and the proportion of agreement. Each pair yielded 10 pairs for comparison. The data for the first measurements were analyzed to evaluate the inter-observer reproducibility.

Analysis of intra-observer reproducibility

The data for the first and second measurements were compared to assess the intra-observer reproducibility for the same observer. The intra-observer reproducibility for the same observer yielded five pairs for comparison.

Statistics

Kappa statistics were performed to determine the concordance/agreement of the tumor response criteria. The potential kappa values ranged from −1.0 (complete disagreement) through 0 (chance agreement) to 1.0 (complete agreement). Interpretations of the strength of the agreement determined using the kappa values were given by adopting the criteria (9). The kappa values of the two agreements were compared for statistical significance using a paired *t* test. Comparisons between groups were done using the Fisher exact test. A conventional *P* value of 0.05 was considered statistically significant. All analyses were conducted using SPSS (version 17.0).

Results

Patient population

Sixty-five untreated lesions in 21 patients treated using pan-hepatic TACE were evaluated. The patients' characteristics were as follows (Table I), median age (range): 68 years (27–74 years); sex (male/female): 19/2; hepatitis C virus/hepatitis B virus/others: 12/3/6; Child–Pugh A/B: 20/1; total number of nodules (range): 65 nodules (1–5 nodules); mean tumor size (range): 20 mm (10–132 mm).

Inter-criteria reproducibility

The inter-criteria reproducibility using RECIST version 1.1 and mRECIST criteria is summarized in Tables II and III. Five observers measured 65 lesions independently, for a total of 325 measurements. According to RECIST version 1.1 criteria, the CR rate and the response rate were 9.2% and 43.1%, respectively; according to mRECIST criteria, the CR rate and the response rate were 56.9% and 79.7% (Table II).

Among the 185 CR lesions that were identified using mRECIST criteria, RECIST version 1.1 criteria classified the same responses as PR for 89 lesions, SD for 64 lesions, and PD for 2 lesions (Table III). The kappa value was 0.149 (95% CI 0.098–0.201), and the proportion of agreement was 35.5% (Table III).

Table I. Patients and characteristics.

No. of patients	21
Age, median (range)	68 (27–74)
Sex (male/female)	19/2
HCV/HBV/others	12/3/6
Child–Pugh A/B	20/1
No. of nodules, all (range)	65 (1–5)
Mean tumor size (range), mm	20 (10–132)

HCV = hepatitis C virus; HBV = hepatitis B virus.

Inter-observer reproducibility

The inter-observer reproducibility among the five observers was analyzed using the data for the first measurements, with each pair yielding 10 pairs for comparison. These 10 pairs for comparisons, or 650 measurements, are collectively shown in Table IV. For the inter-observer reproducibility for RECIST version 1.1, the kappa value was 0.628 (95% CI 0.571–0.684), and the proportion of agreement was 78.8%. For the inter-observer reproducibility for mRECIST, the kappa value was 0.829 (95% CI 0.792–0.866), and the proportion of agreement was 90.0%.

Intra-observer reproducibility

The intra-observer reproducibility was analyzed from the data for the first and second measurements, with each pair yielding five pairs for comparison. These five pairs for comparisons, or 325 measurements, are collectively shown in Table V. For the intra-observer reproducibility for RECIST version 1.1, the kappa value was 0.643 (95% CI 0.565–0.722), and the proportion of agreement was 79.4%. For the intra-observer reproducibility for mRECIST, the kappa value was 0.900 (95% CI 0.858–0.942), and the proportion of agreement was 94.2%.

Discussion

The inter-criteria reproducibility study between RECIST version 1.0 and EASL guidelines, and a comparative study of tumor response by RECIST and mRECIST have been reported (8,9). However, no information is available concerning the inter-observer reproducibility in those reports. In addition to performing an inter-criteria reproducibility study, we also estimated the inter- and intra-observer reproducibility to investigate which set of criteria (RECIST version 1.1 or mRECIST) is superior for performing tumor response evaluations in clinical trials of TACE for HCC.

Inter-criteria reproducibility

An evaluation of the tumor response according to RECIST version 1.0 and EASL guidelines after loco-regional therapies in patients with HCC has been reported. RECIST missed all the CRs obtained by

Table II. Inter-criteria reproducibility between RECIST version 1.1 and mRECIST criteria. Number of lesions (%).

Response category	Complete response	Partial response	Stable disease	Progressive disease	Overall response[a]
Response criteria					
RECIST	30 (9.2)	110 (33.8)	180 (55.4)	5 (1.5)	140 (43.1)
	$P < 0.001$				$P < 0.001$
mRECIST	185 (56.9)	74 (22.8)	65 (20)	1 (3)	259 (79.7)

[a]Complete response + partial response.
RECIST = Response Evaluation Criteria in Solid Tumors; mRECIST = modified RECIST.

Table III. Inter-criteria reproducibility between RECIST version 1.1 and mRECIST criteria: distribution chart.

		RECIST				
		Complete response	Partial response	Stable disease	Progressive disease	Total
mRECIST	Complete response	30	89	64	2	185
	Partial response	0	21	53	0	74
	Stable disease	0	0	63	2	65
	Progressive disease	0	0	0	1	1
Total		30	110	180	5	325

Proportion of agreement = 35.5%. Kappa = 0.149.

tumor necrosis and underestimated the extent of the partial tumor response because of tissue necrosis (8).

In our inter-criteria reproducibility study comparing RECIST version 1.1 and mRECIST criteria, similar results were obtained. The CR rate and the response rate obtained using mRECIST criteria were higher than those obtained using RECIST version 1.1 criteria (56.9% versus 9.2%, $P < 0.001$; 79.7% versus 43.1%, $P < 0.001$).

According to mRECIST criteria, if a tumor that was solid at baseline became entirely necrotic, all the tumors were evaluated as CR. On the other hand, using RECIST version 1.1 criteria, the necrotic tumor was evaluated as a non-CR based on the measurement of the entire lesion, leading to a different conclusion, such as PR, SD, or PD (Figure 2). Among 185 CR lesions that were identified using mRECIST criteria, 155 lesions (83.8%) were evaluated as non-CR using RECIST version 1.1 criteria. In particular, two lesions evaluated as CR using mRECIST criteria were categorized as PD using RECIST version 1.1 criteria; thus, two sets of criteria produced opposite conclusions (Table III). As the tumor size was very small and a 20% increase was thought to be within the range of measurement error, these two lesions were identified as PD using RECIST version 1.1 criteria. In some cases, this event might be caused by an increase in the necrotic tumor size secondary to chemoembolization. Therefore, the inter-criteria reproducibility between RECIST version 1.1 and mRECIST criteria for loco-regional therapy achieving complete tumor necrosis may have a low concordance.

The differences in the CR rate and the response rate between RECIST version 1.1 and mRECIST criteria indicate that the researchers should ascertain the presence or absence of 'm' (mRECIST? or RECIST?).

Table IV. Inter-observer reproducibility.

	Kappa	Proportion of agreement (%)
Inter-observer reproducibility		
RECIST	0.628 (95% CI 0.571–0.684)	78.8
mRECIST	0.829 (95% CI 0.792–0.866)	90.0

Inter- and intra-observer reproducibility

Standardized tumor response evaluation systems are considered to be reliable in clinical trials when they are reproducible among different observers. The importance of inter-observer reproducibility for any

Table V. Intra-observer reproducibility.

	Kappa	Proportion of agreement (%)
Intra-observer reproducibility		
RECIST	0.643 (95% CI 0.565–0.722)	79.4
mRECIST	0.900 (95% CI 0.858–0.942)	94.2

classification scheme has been discussed previously for other grading systems (10-14). Clinical investigators must take into account inter-observer reproducibility in tumor response evaluations, which can greatly affect the results of clinical trials.

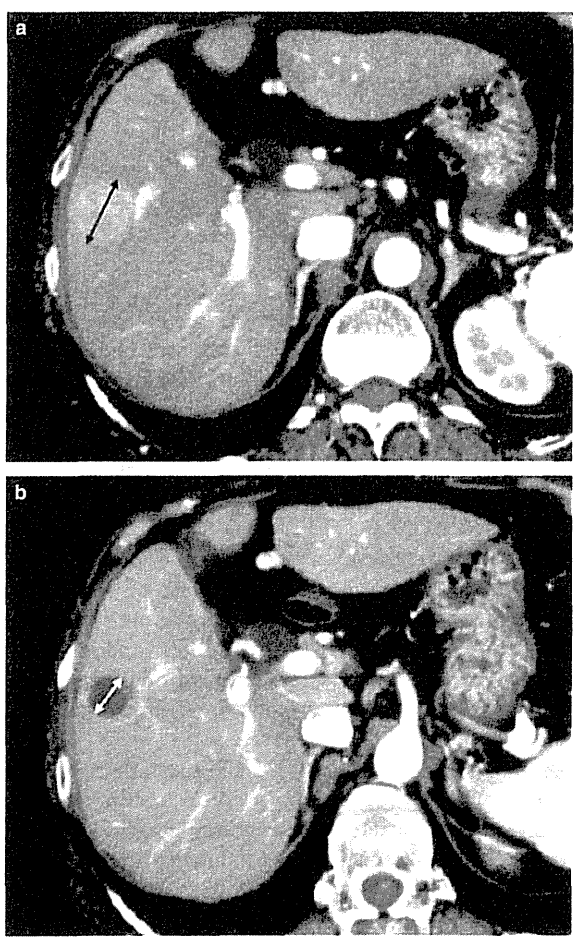

Figure 2. A: CT before TACE: Both criteria (RECIST version 1.1 and mRECIST) measured the longest diameter of the tumor. B: CT after TACE: The tumor had become entirely necrotic. The tumor response was evaluated as CR using mRECIST criteria (i.e. no measurement) and as non-CR using RECIST version 1.1 criteria (i.e. the measurement of the longest diameter of the entire tumor).

In our inter- and intra-observer reproducibility study, the kappa value and the proportion of agreement using mRECIST criteria ('almost perfect agreement') were higher than those for RECIST version 1.1 criteria ('substantial agreement'). In consideration of the high inter- and intra-observer reproducibility, mRECIST can be more recommended for use as tumor response criteria in clinical trials of TACE for HCC.

The present study had several limitations. The number of patients was relatively small, and the analyses were performed not on a per-patient basis, but on a per-lesion basis. To investigate which set of criteria was superior as tumor response criteria in clinical trials of TACE for HCC, the observer consistency study (inter- and intra-observer reproducibility between the two updated sets of criteria) were investigated in this study. A validation study comparing the updated criteria to the gold standard (i.e. overall survival) should be encouraged in future studies.

In conclusion, considering the differences in the CR rate and the response rate between RECIST version 1.1 and mRECIST criteria, close attention must be paid to the criteria used for a precise interpretation of the tumor response outcome. Furthermore, mRECIST criteria may be more suitable for tumor response criteria in clinical trials of TACE for HCC, compared with RECIST version 1.1 criteria, from the viewpoint of the high inter- and intra-observer reproducibility.

Acknowledgements

This study was undertaken as JIVROSG-0602. A part of this study was shown as a poster presentation at the meeting of the Cardiovascular and Interventional Radiological Society of Europe, Lisbon 2009.

Declaration of interest: This work was supported by the Grant-in-Aid for Cancer Research from the Japanese Ministry of Health, Labour and Welfare (20-15). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, 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. 2000;92: 205–16.

2. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis. 2010;30:52–60.
3. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228–47.
4. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001;35:421–30.
5. Varela M, Real MI, Burrel M, Forner A, Sala M, Brunet M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. J Hepatol. 2007;46:474–81.
6. Sala M, Llovet JM, Vilana R, Bianchi L, Solé M, Ayuso C, et al. Initial response to percutaneous ablation predicts survival in patients with hepatocellular carcinoma. Hepatology. 2004;40:1352–60.
7. Llovet JM, Bisceglie AD, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100: 698–711.
8. Forner A, Ayuso C, Varela M, Rimola J, Hessheimer AJ, de Lope CR, et al. Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma. Are response evaluation criteria in solid tumors reliable? Cancer. 2009;115:616–23.
9. Edeline J, Boucher E, Rolland Y, Vauléon E, Pracht M, Perrin C, et al. Comparison of tumor response by response evaluation criteria in solid tumors (RECIST) and modified RECIST in patients treated with sorafenib for hepatocellular carcinoma. Cancer. 2012;118:147–56.
10. Landis JR, Koch GG. The measurement of observer agreement for caterogical data. Biometrics. 1977;33:159–74.
11. Watanabe H, Kunitoh H, Yamamoto S, Kawasaki S, Inoue A, Hotta K, et al. Effect of the introduction of minimum lesion size on interobserver reproducibility using RECIST guidelines in non-small cell lung cancer patients. Cancer Sci. 2006;97: 214–18.
12. Al-Aynati M, Chen V, Salama S, Shuhaibar H, Treleaven D, Vincic L. Interobserver and intraobserver variability using the Fuhrman grading system for renal cell carcinoma. Arch Pathol Lab Med. 2003;127:593–6.
13. Hagen PJ, Hartmann IJ, Hoekstra OS, Stokkel MP, Postmus PE, Prins MH. Comparison of observer variability and accuracy of different criteria for lung scan interpretation. J Nucl Med. 2003;44:739–44.
14. Travis WD, Gal AA, Colby TV, Klimstra DS, Falk R, Koss MN. Reproducibility of neuroendocrine lung tumor classification. Hum Pathol. 1998;29:272–9.

International Journal of
Radiation Oncology
biology • physics

www.redjournal.org

Clinical Investigation: Pancreatic Cancer

A Multicenter Phase II Trial of S-1 With Concurrent Radiation Therapy for Locally Advanced Pancreatic Cancer

Masafumi Ikeda, MD,* Tatsuya Ioka, MD,† Yoshinori Ito, MD,‡ Naohiro Yonemoto, MPH,§ Michitaka Nagase, MD,|| Kenji Yamao, MD,¶ Hiroyuki Miyakawa, MD, Hiroshi Ishii, MD,†† Junji Furuse, MD,‡‡ Keiko Sato, PhD,§§ Tosiya Sato, PhD,|||| and Takuji Okusaka, MD¶¶**

**Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Chiba, Japan; †Department of Hepatobiliary and Pancreatic Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; ‡Department of Radiation Oncology, National Cancer Center Hospital, Tokyo, Japan; §Department of Epidemiology and Biostatistics, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan; ||Department of Clinical Oncology, Jichi Medical University, Tochigi, Japan; ¶Department of Gastroenterology, Aichi Cancer Center Hospital, Nagoya, Japan; **Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo, Japan; ††Hepatobiliary and Pancreatic Division, Cancer Institute Hospital, Tokyo, Japan; ‡‡Department of Internal Medicine, Medical Oncology School of Medicine, Kyorin University, Tokyo, Japan; §§Kyoto Unit Center, Japan Environment and Children's Study, Kyoto University Graduate School of Medicine, Kyoto, Japan; ||||Department of Biostatistics, Kyoto University School of Public Health, Kyoto, Japan; and ¶¶Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan*

Received Feb 8, 2012, and in revised form Mar 24, 2012. Accepted for publication Mar 27, 2012

Summary

S-1 is the first single anti-cancer agent to be judged non-inferior to gemcitabine in a large-scale, randomized, phase III trial for advanced pancreatic cancer, and it can also act as a radiosensitizer. S-1 with concurrent radiation therapy showed very favorable activity, with mild toxicity in patients with

Purpose: The aim of this trial was to evaluate the efficacy and toxicity of S-1 and concurrent radiation therapy for locally advanced pancreatic cancer (PC).

Methods and Materials: Locally advanced PC patients with histologically or cytologically confirmed adenocarcinoma or adenosquamous carcinoma, who had no previous therapy were enrolled. Radiation therapy was delivered through 3 or more fields at a total dose of 50.4 Gy in 28 fractions over 5.5 weeks. S-1 was administered orally at a dose of 80 mg/m^2 twice daily on the day of irradiation during radiation therapy. After a 2- to 8-week break, patients received a maintenance dose of S-1 (80 mg/m^2/day for 28 consecutive days, followed by a 14-day rest period) was then administered until the appearance of disease progression or unacceptable toxicity. The primary efficacy endpoint was survival, and the secondary efficacy endpoints were progression-free survival, response rate, and serum carbohydrate antigen 19-9 (CA19-9) response; the safety endpoint was toxicity.

Results: Of the 60 evaluable patients, 16 patients achieved a partial response (27%; 95% confidence interval [CI], 16%-40%). The median progression-free survival period, overall survival period, and 1-year survival rate of the evaluable patients were 9.7 months (95% CI, 6.9-11.6 months),

Reprint requests to: Masafumi Ikeda, MD, Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital E, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan. Tel: 81-4-7133-1111; Fax: 81-4-7133-0335; E-mail: masikeda@east.ncc.go.jp

This work was presented in abstract form on June 4-6, 2010. at the 46th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL.

This study was supported in part by grants-in-aid for cancer research from the Ministry of Health, Labor, and Welfare of Japan.

Conflict of interest: none.

Supplementary material for this article can be found at www.redjournal.org.

Int J Radiation Oncol Biol Phys, Vol. 85, No. 1, pp. 163–169, 2013
0360-3016/$ - see front matter
doi:10.1016/j.ijrobp.2012.03.059

locally advanced pancreatic cancer.

16.2 months (95% CI, 13.5-21.3 months), and 72% (95%CI, 59%-82%), respectively. Of the 42 patients with a pretreatment serum CA19-9 level of ≥100 U/ml, 34 (81%) patients showed a decrease of greater than 50%. Leukopenia (6 patients, 10%) and anorexia (4 patients, 7%) were the major grade 3-4 toxicities with chemoradiation therapy.

Conclusions: The effect of S-1 with concurrent radiation therapy in patients with locally advanced PC was found to be very favorable, with only mild toxicity. © 2013 Elsevier Inc.

Introduction

Pancreatic cancer (PC), one of the most lethal human cancers, has become the fifth most common cause of death due to cancer in Japan; it has been estimated that PC was responsible for 26,791 deaths in 2009, representing approximately 3% of all deaths. PC patients have a dismal prognosis, as their 5-year survival after diagnosis is less than 5%. Of all treatment modalities available for PC, only resection offers an opportunity for a cure. However, approximately half of patients already have metastases at the time of diagnosis, and approximately one-third of patients are diagnosed as having locally advanced disease, whereas only a small proportion of patients are eligible for surgery, as a result of the lack of effective screening. Concurrent chemoradiation therapy with external beam radiation therapy and chemotherapy using 5-fluorouracil (5-FU) is often used in patients who have unresectable PC due to vascular involvement that includes the celiac artery or supra-mesenteric artery, with no distant metastases on radiological examination, because it is generally accepted as a standard therapy for locally advanced PC (1-4). A variety of anticancer agents, including gemcitabine (5) and capecitabine (6), and various radiation schedules (7-8) have been examined in clinical trials, but survival has not been significantly improved.

S-1 is a new oral fluoropyrimidine derivative in which tegafur is combined with 2 5-chloro-2,4-dihydroxypyridine modulators and oteracil potassium, a potentiator of 5-FU's antitumor activity that also decreases gastrointestinal toxicity. A multi-institutional, late-phase II trial of S-1 involving metastatic PC patients reported a good tumor response rate (38%) and improved survival (median, 9.2 months) (9). A phase III trial compared therapy with S-1, with gemcitabine alone, and with gemcitabine plus S-1 in patients with unresectable PC in Japan and Taiwan, and S-1 therapy was found to provide efficacy and toxicity similar to gemcitabine when it was used as a first-line treatment for advanced PC (median survival: S-1, 9.7 months; gemcitabine, 8.8 months [hazard ratio, 0.96; non-inferiority P value <.001]); thus, S-1 was judged to be non-inferior to gemcitabine (10). S-1 also acts as a radiosensitizer, and preclinical and clinical studies have demonstrated the radiosensitizing potency of S-1 (11). Not only is S-1 a potent radiosensitizer that has been shown to have promising antitumor activity against advanced PC, but also, since it is active orally, it is also much more convenient for patients than intravenous 5-FU infusion. Thus, concurrent raditation therapy and oral S-1 instead of 5-FU infusion may be a more efficient treatment that also improves patients' quality of life. In a phase I trial conducted in one of our hospitals, the recommended S-1 dose with concurrent radiation therapy was found to be 80 mg/m^2/day on the day of irradiation; at this dose, S-1 was found to have excellent antitumor activity with mild toxicity (12). Consequently, a multi-institutional phase II study was conducted to clarify the efficacy and safety of concomitant radiation therapy with S-1 in patients with locally advanced PC.

Methods and Materials

Patients and eligibility

Patients eligible for study entry had locally advanced nonresectable clinical stage III (T4N0-1 and M0) PC, according to International Union Against Cancer criteria. Eligibility criteria were adenocarcinoma or adenosquamous carcinoma confirmed on cytology or histology; no previous chemotherapy for PC; a square (10 cm × 10 cm) radiation field could encompass all pancreatic lesions and lymph node metastases; age ≥20 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0-2; adequate oral intake; satisfactory hematological functions (hemoglobin concentration, ≥9.0 g/dl; leukocyte count, ≥3500/mm^3; platelet count, ≥100,000/mm^3); adequate hepatic function (serum total bilirubin ≤2.0 times the upper normal limit [UNL] or ≤3.0 mg/dl with biliary drainage); aspartate aminotransferase [AST] and alanine aminotransferase [ALT] ≤2.5 times UNL or ≤5 times UNL with biliary drainage; serum albumin ≥3.0 g/dl; and normal renal function (serum creatinine ≤UNL). Written informed consent was obtained from all patients.

Exclusion criteria were active infection; active gastroduodenal ulcer; watery diarrhea; phenytoin, warfarin potassium, or flucytosine treatment; pleural effusion or ascites; severe complications such as cardiac or renal disease; psychiatric disorder; history of drug hypersensitivity; and active concomitant malignancy. In addition, pregnant and lactating women and women of childbearing age who were not using effective contraception were also excluded.

Pretreatment evaluation required a complete history and physical examination and baseline assessments of organ function. In addition, contrast medium-enhanced computed tomography (CT) or magnetic resonance imaging of the abdomen and X-ray or CT of the chest was performed for pretreatment staging to assess the local extension of the tumor and to exclude the presence of distant metastases. The criteria for local extension surrounding the pancreas included tumor invasion to the celiac trunk or superior mesenteric artery, or both, which corresponded to clinical stage III according to the International Union Against Cancer (6th edition). All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. Laparoscopy and laparotomy to rule out occult peritoneal dissemination prior to study entry were not necessary.

Treatment schedule

The regimen consisted of S-1 with concurrent radiation therapy and maintenance S-1 chemotherapy.

S-1 with concurrent radiation therapy

Radiation therapy was delivered with >6-MV photons, using a multiple (three or more) field technique. A total dose of 50.4 Gy

was delivered in 28 fractions over 5.5 weeks. Primary tumor and metastatic lymph nodes >1 cm identified on CT were contoured as gross tumor volumes (GTV). The clinical target volume (CTV) included the primary tumor with a 0.5-cm margin and metastatic lymph nodes. Regional lymph nodes were not treated electively. The definition of planning target volume (PTV) include the CTV with a 1-cm margin laterally and a 1- to 2-cm margin in the craniocaudal direction to take into account respiratory organ motion and daily set-up errors. The reference point for the radiation dose was set at the center of the PTV. The spinal cord dose was maintained at <45 Gy. The volume of liver to receive 30 Gy was required to be <40%, and the volume to receive 20 Gy was required to be <67%. At least 75% of both kidneys was required to receive less than 18 Gy.

S-1 was administered orally at a dose of 40 mg/m^2 twice daily after breakfast and dinner on the day of irradiation (Monday through Friday) during radiation therapy. The 3 initial doses were determined according to the body surface area (BSA) as follows: patients with a BSA of <1.25 m^2 received 40 mg/dose; those with BSA of 1.25 m^2-<1.5 m^2 received 50 mg/dose; and those with BSA of ≥1.5 m^2 received 60 mg/dose. The dose of S-1, which is the standard dose when S-1 is used as a single agent for systemic therapy (15, 16), had been previously determined in our phase I trial (19).

The occurrence of grade 4 hematological toxicity, grade 3 non hematological toxicity excluding nausea, anorexia, fatigue, constipation, and hyperglycemia, or a serum AST or ALT >200 IU/l resulted in the suspension of radiation therapy and S-1 administration. When the toxicities improved by at least 1 grade compared to the suspension criteria, treatment was resumed. When suspension criteria were met, dose modification was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of 1.25 m^2-<1.5 m^2 received 40 mg/dose; and those with a BSA ≥1.5 m^2 received a 50 mg/dose. Chemoradiation therapy was discontinued when the patient developed grade 4 non-hematological toxicities or other unacceptable toxicities, including gastrointestinal ulcer or bleeding, interruptions in treatment of >15 days, or unequivocal tumor progression. After treatment discontinuation, patients could receive other anticancer treatments excluding S-1 with concurrent radiation therapy at their physician's discretion.

Maintenance S-1 chemotherapy

From 2-8 weeks after completion of S-1 with concurrent radiation therapy, maintenance S-1 chemotherapy was initiated at a dose of 40 mg/m^2 twice daily orally, after breakfast and dinner, for 28 consecutive days, followed by a 14-day rest period per course. 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, temporary interruption or dose reduction of S-1 administration was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of ≤1.25 m^2-<1.5 m^2 received a 40 mg/dose; and those with a BSA of ≥1.5 m^2 received a 50 mg/dose. When grade 4 non hematological toxicities, unacceptable toxicities, a rest period >28 days, or an unequivocal tumor progression was observed during maintenance S-1 chemotherapy, treatment was discontinued. After treatment discontinuation, patients could be given other anticancer treatment, excluding S-1 monotherapy, at their physician's discretion.

Response and toxicity assessment

Evaluations of tumor response during chemoradiation therapy and maintenance therapy were performed at the completion of chemoradiation therapy and every 6 weeks thereafter until tumor progression or 24 weeks from the start of S-1 and radiation therapy, using the Response Evaluation Criteria in Solid Tumors version 1.0 questionnaire. Responses were evaluated centrally by 3 independent reviewers. Serum carbohydrate antigen 19-9 (CA19-9) levels were measured at least every 6 weeks. In patients with a pretreatment CA19-9 level ≥100 U/ml, the CA19-9 response was assessed; a positive response was defined as a reduction of >50% from the pretreatment level (13). Overall survival was measured from the date of initial treatment to the date of death or the date of the last follow-up. Progression-free survival was defined as the time from the date of initial treatment to the first documentation of progression or death. Basic laboratory tests that included a complete blood count with differentials, serum chemistry, and urinalysis were administered at least weekly during S-1 therapy and radiation therapy and then at least once every 2 weeks during S-1 maintenance therapy. Common Terminology Criteria for Adverse Events, version 3.0, were used for the assessment of treatment-related toxicities.

Radiation therapy quality assurance

All radiation therapy treatment plans for the enrolled patients were reviewed centrally by an independent radiation committee consisting of 9 radiation oncologists. To assess radiation therapy protocol compliance, the following parameters were reviewed: fraction size, prescribed dose to the reference point, energy, relationships between GTV, CTV, PTV and radiation field, overall treatment time, isodose distributions at the transverse section of the reference points, and doses to organs at risk. The quality assurance assessment was given as per protocol (PP), deviation acceptable (DA), and violation unacceptable (VU). After parameter compliance was assessed, overall radiation therapy compliance was classified as: PPoverall, no DA or VU in any parameter; VUoverall, at least 1 VU in any parameter; or DAoverall, neither PP nor VU.

Statistical considerations

Primary endpoints of this trial were overall survival for the efficacy evaluation and frequency of adverse events for the safety evaluation; secondary endpoints were progression-free survival, response rate, and serum CA19-9 level response.

The enrollment goal was set at 60 eligible patients. The number of enrolled patients was determined using a statistical power analysis. Under the assumptions of a median survival time of 10 months for patients receiving conventional chemoradiation therapy (1-4), a 2-year registration period followed by a 2-year follow-up period and a one-sided alpha level of 5%, the statistical power of the hazard ratio test was over 70% or 90% with the expected median survival time of 14 or 16 months, respectively. Therefore, the number of planned enrolled patients, the registration period, the follow-up period, and the total research period were set at 60, 2 years, 2 years, and 4 years, respectively. The full analysis set (FAS) was defined as any patient who received at least 1 course of study medication. Overall and progression-free survival curves were calculated using the Kaplan-Meier method. This open-label, multi-institutional, single arm

phase II study was approved by the review board of each institution and was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Clinical Research (Ministry of Health, Labour, and Welfare, Japan). The trial was registered at University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index-j.htm), identification number (UMIN000000486).

Patient registration and data collection were managed by the Makimoto-han datacenter. The quality of the data was ensured by a careful review performed by the data center staff and the coordinating investigator of this study (MI). All data were fixed on November 13, 2009, and all analyses in this study were performed by statisticians (NY and TS).

Results

Patient characteristics

Sixty-one patients were enrolled in this trial between July 2006 and November 2007 at 20 institutions in Japan (see the Appendix in Supplementary Material). However, 1 patient was excluded before the start of protocol treatment because distant lymph node metastases were detected during a CT examination for radiation field planning; this patient received systemic chemotherapy with gemcitabine alone. Table 1 shows the characteristics of the 60 FAS patients.

Table 1 Patient characteristics (n=60)

Characteristics	No. of patients	Value(s)	% of patients
Age (y)			
Median		64	
Range		31-80	
Sex			
Male	35		58
Female	25		42
Eastern Cooperative Oncology Group performance status			
0	34		57
1	26		43
Biliary drainage			
Present	16		27
Pathology			
Adenocarcinoma	59		98
Adenosquamous carcinoma	1		2
Tumor location			
Head	33		55
Body or tail	27		45
Maximum tumor size, cm			
Median		3.6	
Range		2.0-6.5	
Regional lymph node swelling			
N0	44		73
N1	16		27
CA19-9 (U/ml)			
Median		304	
Range		0-4400	
Planning target volume (cm^3)			
Median		240	
Range		102-442	

Abbreviation: CA19-9 = carbohydrate antigen 19-9.

Fifty-three patients (88%) completed S-1 therapy and radiation therapy but the remaining 7 patients (12%) discontinued S-1 and radiation therapy. Reasons for treatment discontinuation were disease progression (2 patients), duodenal and bile duct perforation (1 patient), acute myocardial infarction (1 patient), treatment interruption for >15 days because of cholangitis (1 patient), severe confusion (1 patient), and patient refusal to continue treatment because of grade 3 nausea and vomiting (1 patient). The treatment delay during chemoradiation therapy was observed in 20 patients (33%), and the median delay was 3 days (range, 1-17 days). Compliance with S-1 therapy was high, with a rate of 99% (1170 of 1176 doses). Of the 53 patients who completed chemoradiation therapy 47 (89%) patients received maintenance S-1 chemotherapy, but 6 patients did not for the following reasons: disease progression (3 patients); sudden death because of septic shock of unknown origin occurring 40 days after the completion of S-1 and radiation therapy (1 patient); and patient refusal to continue treatment because of grade 2 nausea and grade 2 diarrhea (1 patient) or grade 3 appetite loss and grade 2 fatigue (1 patient). The median number of S-1 maintenance chemotherapy courses was 4 (range, 1 to ≥19). At the time of the final analysis, S-1 maintenance chemotherapy had been terminated in 46 (98%) of 47 patients because of disease progression (29 patients, 63%), adverse events (12 patients, 26%), patient refusal (2 patients, 4%), or other reasons (3 patients, 7%). Treatment delay during the first and second courses of maintenance S-1 therapy was observed in 9 patients (19%) and 7 patients (18%), respectively. The rate of compliance with S-1 chemotherapy was 91% (2503 of 2744 doses) in the first course and 98% (2149 of 2184 doses) in the second course. After the completion of protocol treatment, 53 patients (88%) received subsequent therapy including gemcitabine (47 patients), S-1 (11 patients), radiation therapy for bone metastases (2 patients), and other treatments (4 patients).

Toxicity

The toxicities of S-1 and radiation therapy observed in the 60 FAS patients are listed in Table 2. Grade 3 leukocytopenia, neutropenia, and anemia occurred in 6 (10%), 3 (5%), and 2 (3%) patients, respectively; no grade 4 hematological toxicity was seen. The most common and troublesome non-hematological toxicities for patients undergoing chemoradiation therapy were usually gastrointestinal toxicities, including anorexia, nausea, and vomiting. However, grade 3 or higher cases of these toxicities were observed only in 4 (7%), 3 (5%), and 2 (3%) patients, respectively, and the toxicities were generally mild and manageable. One treatment-related death arising from perforation of the duodenum and biliary tract occurred during chemoradiation therapy.

Toxicities occurring during S-1 maintenance chemotherapy were also mild and transient (Table 3). Grade 4 leukocytopenia was the only hematological toxicity, and it was observed in only 1 patient (2%); the incidence of grade 3 or higher gastrointestinal toxicities was <6%. In addition, no serious adverse events occurred during S-1 maintenance chemotherapy. No late toxicities that could be associated with S-1 and radiation therapy were reported.

Efficacy

The response evaluation included all 60 FAS patients, but tumor response was not evaluable in 1 patient in whom contrast-enhanced CT examination could not be performed due to deterioration of her general condition following duodenal perforation.

Table 2 Toxicity during S-1 and concurrent radiation therapy (n=60)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	15 (25)	28 (47)	6 (10)	0 (0)
Neutrophils	9 (15)	15 (25)	3 (5)	0 (0)
Hemoglobin	16 (27)	13 (22)	2 (3)	0 (0)
Platelets	24 (40)	3 (5)	0 (0)	0 (0)
Non hematological				
Rash	2 (3)	0 (0)	0 (0)	0 (0)
Pigmentation	6 (10)	0 (0)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Gastric ulcer/gastritis	0 (0)	1 (2)	1 (2)	0 (0)
Abdominal pain	0 (0)	0 (0)	1 (2)	0 (0)
Bilirubin	4 (7)	1 (2)	1 (2)	0 (0)
Aspartate aminotransferase	11 (18)	3 (5)	0 (0)	0 (0)
Alanine aminotransferase	10 (17)	5 (8)	0 (0)	0 (0)
Alkaline phosphatase	4 (7)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	15 (25)	7 (12)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	0 (0)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (3)	4 (7)	0 (0)	0 (0)
Cholangitis	0 (0)	1 (2)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

Table 3 Toxicity during S-1 maintenance therapy (n=47)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	4 (9)	27 (57)	4 (9)	1 (2)
Neutrophils	5 (11)	19 (40)	6 (13)	0 (0)
Hemoglobin	8 (17)	18 (38)	3 (6)	0 (0)
Platelets	8 (17)	2 (4)	1 (2)	0 (0)
Non hematological				
Malaise	13 (27)	8 (17)	2 (4)	0 (0)
Anorexia	15 (32)	11 (23)	3 (6)	0 (0)
Nausea	7 (15)	4 (9)	1 (2)	0 (0)
Vomiting	4 (9)	1 (2)	0 (0)	0 (0)
Diarrhea	3 (6)	3 (6)	0 (0)	0 (0)
Stomatitis	4 (9)	0 (0)	0 (0)	0 (0)
Alopecia	1 (2)	0 (0)	-	-
Rash	2 (4)	1 (2)	0 (0)	0 (0)
Pigmentation	11 (23)	1 (2)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Duodenal ulcer	0 (0)	1 (2)	0 (0)	0 (0)
Taste alteration	1 (2)	2 (4)	-	-
Bilirubin	7 (15)	5 (11)	0 (0)	0 (0)
Aspartate aminotransferase	8 (17)	3 (6)	1 (2)	0 (0)
Alanine aminotransferase	5 (11)	2 (4)	0 (0)	0 (0)
Alkaline phosphatase	1 (2)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	10 (21)	5 (11)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	3 (6)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (4)	4 (9)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

Tumor response was evaluated based on the best response as of 24 weeks after S-1 and radiation therapy were started. Overall, a partial response was seen in 16 patients for an overall response rate of 27% (95% confidence interval [CI], 16%-40%). The median survival in patients with partial response was 19.4 months (range, 9.8-32.6 months; 95% CI, 13.9-25.1 months), with a median duration of response of 7.3 months (range, 5.5-10.1 months). Forty patients (67%) showed stable disease, and 3 patients (5%) had progressive disease. Additionally, tumor response was evaluated for all periods because tumor shrinkage was obtained in some patients after 24 weeks. Of the 40 patients who were judged to have stable disease on the response evaluation at 24 weeks, an additional 6 patients were judged to have a partial response by the central independent reviewers. The median time to partial response was 4.7 months (range, 1.4-16.8 months) after chemoradiation therapy commenced. Therefore, the response rate for all periods was 37% (95% CI, 25%-50%). Of the 42 patients with a pretreatment serum CA19-9 level ≥100 U/ml, 34 (81%) patients had a >50% decrease compared to the pretreatment level. During this protocol treatment, 2 patients underwent surgical resection because tumor shrinkage occurred and their tumors became resectable.

Fifty-four of the 60 patients had disease progression at the time of the analysis. The median progression-free survival time and the 6-month and 1-year progression-free survival proportions for all patients were 9.7 months (95% CI, 6.9-11.6 months), 68%, and 32%, respectively (Fig.). The pattern of disease progression was distant metastases in 26 patients (46%), locoregional recurrence in 16 patients (27%), distant metastases and locoregional recurrence in 3 patients (5%), and deterioration of general condition in 9 patients (15%). At the time of analysis, 49 patients had died, and the median follow-up period was 16.3 months (range, 3.0-34.0 months). The median survival time and the 1-year and 2-year survival proportions for the 60 patients were 16.2 months (95% CI, 13.5-21.3 months), 72% (95% CI, 59%-82%), and 26%, respectively (Fig.).

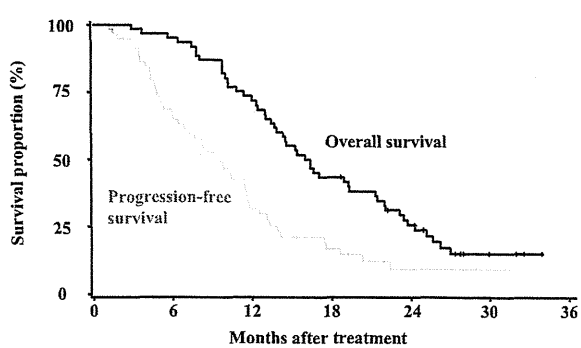

Fig. Overall survival and progression-free survival curves of the 60 locally advanced PC patients treated with S-1 with concurrent radiation therapy. Censored cases are shown by tick marks.

Radiation therapy quality assurance

Radiation therapy quality assurance was reviewed centrally by an independent radiation committee for all 60 FAS patients. DA was observed for 2 parameters in 4 patients (relationship between GTV and radiation field, 2 patients; isodose distribution, 2 patients), but no instances of VU were seen in this study. Therefore, PPoverall, DAoverall, and VUoverall were assessed in 56 (93%) patients, 4 (7%) patients, and 0 (0%) patients, respectively.

Discussion

The combination of radiation therapy and 5-FU chemotherapy has been acknowledged as a standard therapy for locally advanced PC (1-4). However, optimal chemotherapeutic regimens continue to be pursued, as the survival benefit remains modest. S-1 is the first single anticancer agent to be judged non-inferior to gemcitabine in a large-scale randomized phase III trial for advanced PC (10), and it is expected to become a first-line treatment for patients with advanced PC, at least in Asian countries. In addition, it has been shown that combined S-1 and radiation therapy has a synergistic effect against 5-FU-resistant cancer xenografts; thus, S-1 may also have a radiosensitizing effect (11). With S-1 and standard-dose radiation therapy (50.4 Gy/28 fractions), the full dose (80 mg/m^2) of S-1 can be given on the day of irradiation (12) with a reduced risk of distant metastases. Therefore, S-1 may act not only against systemic tumor spread but also a as a potent radiosensitizer to enhance local control. Furthermore, the fact that S-1 can be given orally is an additional benefit over 5-FU infusion.

In the present multicenter trial, the 24-week tumor response rate was 27%, although the overall tumor response rate for the complete period was 37%; in fact, tumor resection was possible in 2 patients after treatment. Thus, excellent tumor shrinkage appears to be an additional benefit of this treatment. Furthermore, other outcomes, including the serum CA19-9 level response (81%), progression-free survival (median, 9.7 months), and overall survival (median, 16.2 months), showed excellent results. As the subsequent therapy, most patients (78%) received gemcitabine, as it might lead to favorable overall survival. However, the outcome of S-1 and concurrent radiation therapy has been reported by other groups (14-16), which were single institutional studies with small numbers of enrolled patients and had slight differences in S-1 administration (Table 4). Similar results were obtained, although such nonrandomized data must be interpreted with caution. Given the recent reports of chemoradiation therapy (4-8, 17, 18), S-1 with concurrent radiation therapy appears to have a favorable treatment efficacy for locally advanced PC, and its survival time will approach that of resected PC patients.

During chemoradiation therapy the major troublesome adverse events were gastrointestinal toxicities (anorexia, nausea, and vomiting), which required intravenous fluid infusion and, sometimes, the termination of chemoradiation therapy (4). One approach to reducing these toxicities that has recently come to be used in chemoradiation therapy using conventional photons for the treatment of PC (4, 6), is a limited radiation field, with a PTV including gross tumor volume alone, without prophylactic nodal irradiation; this minimizes the irradiation of normal tissue and was adopted in the present study. Grade 3 or higher of the above-mentioned toxicities were observed in less than 7% of the patients, and the gastrointestinal toxicities were very mild and easily managed. Other grade 3 or higher non hematological and hematological toxicities of S-1 and concurrent radiation therapy were observed in only 10% or less of the patients and were mild, although there was one treatment-related death due to a perforated duodenum. The toxicities associated with maintenance S-1 therapy were also mild, and this regimen was considered to be well tolerated.

Regarding the results of the radiation therapy quality assurance evaluations performed in this study, 93% of the treatments were assessed as PPoverall; this result is excellent compared with that of a previous trial (5). This result was achieved thanks to the efforts made by the radiation oncologists. The radiation technique that was used in this study was thoroughly explained to all of the radiation oncologists at each institution before patient registration, and the radiation therapy records of the enrolled patients were reviewed by the radiation committee. Results of the review were returned to the radiation oncologists at each institution if any problem with the radiation technique was noted. Therefore, a high quality of radiation therapy was maintained in this study.

There continues to be debate about the role of chemoradiation therapy for patients with locally advanced PC. Prior to the 1990s, it was shown that concurrent external-beam radiation therapy and 5-FU chemotherapy offers a survival benefit over radiation therapy (1, 2) or chemotherapy alone (3). Since the introduction of gemcitabine, which is acknowledged as the first-line therapy for advanced PC, 2 randomized controlled trials comparing chemoradiation therapy with gemcitabine alone have been reported:

Table 4 Results of phase II trials of S-1 and radiation therapy for locally advanced pancreatic cancer

Study (ref.)	Y	Chemotherapy	Radiation therapy	No. of patients	Response rate	Median survival time (mo)	1-y survival rate (%)	Median progression-free survival time (mo)	Maintenance chemotherapy
Kim et al (20)	2008	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	25	24%	12.9	43%	6.5	Gemcitabine-based regimen
Sudo et al (15)	2011	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	34	41%	16.8	70.6%	8.7	S-1
Shinchi et al (16)	2011	S-1, 80 mg/m^2, days 1-21	50 Gy/40 fractions	50	30%	14.3	62%	6.7	S-1
Current study		S-1, 80 mg/m^2, on the day of irradiation	50.4 Gy/28 fractions	60	27%	16.2	72%	9.7	S-1

a French group reported an inferior outcome with radiation therapy plus 5-FU and cisplatin to chemotherapy with gemcitabine alone (17); and the ECOG study demonstrated that radiation therapy plus gemcitabine had a superior survival outcome compared with gemcitabine alone (18). Thus, these 2 recent randomized controlled trials comparing chemoradiation therapy with gemcitabine alone demonstrated opposite survival results, although both trials were terminated halfway through because of poor patient accrual. In addition, gemcitabine monotherapy for locally advanced PC has been reported to have a favorable efficacy (median survival, 15 months) according to our Japanese group (19), although the time to treatment failure (median, 6.0 months) was not optimal. Thus, in patients with locally advanced PC, it is not clear whether chemoradiation therapy or chemotherapy alone has a better outcome, and there is a need for a prospective, randomized, controlled study comparing chemoradiation therapy with chemotherapy in such patients. Recently, induction chemotherapy followed by chemoradiation therapy has been reported (20). The role of induction chemotherapy is to prevent distant metastases and to define a subset of patients who are likely to benefit from chemoradiation therapy excluding patients with chemoresistant and rapidly progressive disease. Further clinical trials are needed to elucidate the usefulness of this therapeutic strategy.

Conclusions

S-1 therapy with concurrent radiation therapy had very favorable activity, with mild toxicity in patients with locally advanced PC, and the survival time of such patients is expected to approach that of resected PC patients. This regimen appears to be a good platform for incorporation of biologic agents, and the present results should be confirmed in a prospective, randomized, controlled study to elucidate whether chemoradiation therapy or chemotherapy alone results in a better treatment outcome.

References

1. Moertel CG, Childs DS, Reitemeier RJ, et al. Combined 5-fluorourail and supervoltage radiation therapy of locally unresectable gastrointestinal cancer. *Lancet* 1969;2:865-867.
2. Moertel CG, Frytak S, Hahn RG, et al. Therapy of locally unresectable pancreatic carcinoma: a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil. *Cancer* 1981; 48:1705-1710.
3. Gastrointestinal Tumor Study Group. Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. *J Natl Cancer Inst* 1988;80:751-755.
4. Huguet F, Girard N, Guerche CS, et al. Chemoradiotherapy in the management of locally advanced pancreatic carcinoma: a qualitative systematic review. *J Clin Oncol* 2009;27:2269-2277.
5. Mamon HJ, Niedzwiecki D, Hollis D, et al., for the Cancer and Leukemia Group B. A phase 2 trial of gemcitabine, 5-florouracil, and radiation therapy in locally advanced nonmetastatic pancreatic adenocarcinoma: Cancer and Leukemia Group B (CALGB) 80003. *Cancer* 2011;117:2620-2628.
6. Crane CH, Winter K, Regine WF, et al. Phase II study of bevacizumab with concurrent capecitabine and radiation followed by maintenance gemcitabine and bevacizumab for locally advanced pancreatic cancer: Radiation Therapy Oncology Group RTOG 0411. *J Clin Oncol* 2009; 27:4096-4102.
7. Mahadevan A, Jain S, Goldstein M, et al. Stereotactic body radiotherapy and gemcitabine for locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2010;78:735-742.
8. Schellenberg D, Kim J, Christman-Skieller C, et al. Single-fraction stereotactic body radiation therapy and sequential gemcitabine for the treatment of locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2011;81:181-188.
9. Okusaka T, Funakoshi A, Furuse J, et al. A late phase II study of S-1 for metastatic pancreatic cancer. *Cancer Chemother Pharmacol* 2008; 61:615-621.
10. Ioka T, Ikeda M, Ohkawa S, 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 [Abstract 4007]. *J Clin Oncol* 2011;29(suppl 4058).
11. Fukushima M, Sakamoto K, Sakata M, et al. Gimeracil, a component of S-1, may enhance the antitumor activity of X-ray irradiation in human cancer xenograft models in vivo. *Oncol Rep* 2010;24:1307-1313.
12. Ikeda M, Okusaka T, Ito Y, et al. A phase I trial of S-1 with concurrent radiotherapy for locally advanced pancreatic cancer. *Br J Cancer* 2007;96:1650-1655.
13. Hess V, Glimelius B, Grawe P, et al. CA 19-9 tumour-marker response to chemotherapy in patients with advanced pancreatic cancer enrolled in a randomised controlled trial. *Lancet Oncol* 2008;9:132-138.
14. Kim HM, Bang S, Park JY, et al. Phase II trial of S-1 and concurrent radiotherapy in patients with locally advanced pancreatic cancer. *Cancer Chemother Pharmacol* 2009;63:535-541.
15. Sudo K, Yamaguchi T, Ishihara T, et al. Phase II study of oral S-1 and concurrent radiotherapy in patients with unresectable locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2011;80:119-125.
16. Shinchi H, Maemura K, Mataki Y, et al. A phase II study of oral S-1 with concurrent radiotherapy followed by chemotherapy with S-1 alone for locally advanced pancreatic cancer. *J Hepatobiliary Pancreat Sci* 2012;19:152-158.
17. Chauffert B, Mornex F, Bonnetain F, et al. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. *Ann Oncol* 2008;19:1592-1599.
18. Loehrer PJ Sr., Feng Y, Cardenes H, et al. Gemcitabine alone versus gemcitabine plus radiotherapy in patients with locally advanced pancreatic cancer: an Eastern Cooperative Oncology Group Trial. *J Clin Oncol* 2011;29:4105-4112.
19. Ishii H, Furuse J, Boku N, et al. Phase II study of gemcitabine chemotherapy alone for locally advanced pancreatic carcinoma: JCOG 0506. *Jpn J Clin Oncol* 2010;40:573-579.
20. Krishnan S, Rana V, Janjan NA, et al. Induction chemotherapy selects patients with locally advanced, unresectable pancreatic cancer for optimal benefit from consolidative chemoradiation therapy. *Cancer* 2007;110:47-55.

FULL PAPER

BJC

British Journal of Cancer (2013) 108, 2063–2069 | doi: 10.1038/bjc.2013.174

Keywords: pancreatic cancer; gemcitabine; interleukin-1β; interleukin-6; chemotherapy

Serum levels of IL-6 and IL-1β can predict the efficacy of gemcitabine in patients with advanced pancreatic cancer

S Mitsunaga*[,1,2], M Ikeda[1], S Shimizu[1], I Ohno[1], J Furuse[3], M Inagaki[4], S Higashi[5], H Kato[5], K Terao[6] and A Ochiai[2]

[1]*Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan;* [2]*Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan;* [3]*Department of Medical Oncology, Kyorin University School of Medicine, Mitaka, Japan;* [4]*Center for Suicide Prevention, National Institute of Mental Health, National Center of Neurology and Psychiatry, Kodaira, Japan;* [5]*Primary Lifecycle Management Department, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan and* [6]*Clinical Research Planning Department, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan*

Background: With this study, we sought to characterise the impact of pro-inflammatory cytokines on the outcomes of gemcitabine monotherapy (GEM) in patients with pancreatic cancer (PC).

Methods: Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment. All of the patients were scheduled to undergo systemic chemotherapy. Serum pro-inflammatory cytokines were measured using an electro-chemiluminescence assay method before chemotherapy. High cytokine levels were defined as values greater than the median. Clinical data were collected prospectively.

Results: Sixty patients who received GEM were included in the analysis. High IL-6 and IL-1β levels were poor prognostic factors for overall survival in a multivariate analysis ($P=0.011$ and $P=0.048$, respectively). Patients with both a high IL-6 level and a high IL-1β level exhibited shortened overall and progression-free survival, a reduction in the tumour control rate, and a high dose intensity of GEM compared with patients with low levels of both IL-6 and IL-1β.

Conclusion: The serum levels of IL-6 and IL-1β predict the efficacy of GEM in patients with advanced PC.

An increase in inflammatory markers is associated with poor prognosis in patients receiving systemic chemotherapy for advanced pancreatic cancer (PC) (Tanaka *et al*, 2008; Morizane *et al*, 2011). C-reactive protein (CRP) is an index of systemic inflammation that is synthesised in hepatocytes by pro-inflammatory cytokines, including IL-1β (Young *et al*, 2008), IL-6 (Morrone *et al*, 1988), IL-8 (Wigmore *et al*, 1997), and TNF-α (Ganapathi *et al*, 1998), via the transcription factor nuclear factor-κB (NF-κB) and the activation of the signal transducer and activator of transcription 3 (STAT3) protein (Nishikawa *et al*, 2008). NF-κB and STAT3 represent major inflammatory pathways for pro-inflammatory cytokines and contribute to the chemoresistance of tumours (Aggarwal *et al*, 2009). An increase in the effects of pro-inflammatory cytokines is believed to attenuate the benefits of chemotherapy and to result in a poor outcome. Recently, the efficacy of anti-inflammatory therapy has been reported in several diseases: with canakinumab as an IL-1β blocker in the cryopyrin-associated periodic syndrome (Kuemmerle-Deschner *et al*, 2011), with tocilizumab as an IL-6 receptor blocker in rheumatoid arthritis (Jones *et al*, 2010), and with siltuximab as an IL-6 blocker in prostate cancer (Dorff *et al*, 2010). In the blockade of intracellular pathways, ruxolitinib is a Janus kinase inhibitor that

*Correspondence: Dr S Mitsunaga; E-mail: smitsuna@east.ncc.go.jp

Received 18 February 2013; revised 15 March 2013; accepted 24 March 2013; published online 16 April 2013

suppresses STAT3 phosphorylation and has shown clinical benefits in myelofibrosis (Harrison *et al*, 2012). The potential for individual pro-inflammatory cytokines to decrease chemotherapeutic efficacy suggests that it may be a candidate for testing anti-inflammatory therapy in advanced PC patients. This study sought to characterise the impact of pro-inflammatory cytokines on the outcomes of systemic chemotherapy in patients with advanced PC.

MATERIALS AND METHODS

Patients. Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment in this study. Pathological confirmation was obtained from all the patients via either a fine-needle aspiration biopsy or a cytological examination. All the patients were scheduled to undergo chemotherapy at the National Cancer Center Hospital East. A serum sample was obtained on the morning before chemotherapy and was frozen at −70 °C until analysis. Clinical data were prospectively collected before chemotherapy, at 1 month after chemotherapy, and every 3 months after the start of chemotherapy. The tumour stage was evaluated according to the seventh criteria of the International Union Against Cancer (UICC) (Sobin *et al*, 2009). This study was approved by the National Cancer Center Ethics Committee, and patients who provided written informed consent were examined.

Systemic chemotherapy. Gemcitabine monotherapy (GEM) and GEM-based regimens were conducted according to previous reports (Ioka *et al*, 2011; Kindler *et al*, 2011). Most of the patients were scheduled to receive GEM as follows: 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 the occurrence of disease progression, unacceptable toxicity, or patient refusal. The dose intensity of GEM was calculated during the treatment interval between the date of the first administration and the date of the last administration. The planned dose intensity of GEM for a 28-day cycle was 750 mg m^{-2} per week.

Assessment of the anti-tumour effect. The anti-tumour effect of the systemic chemotherapy was evaluated using contrast computed tomography/magnetic resonance imaging images obtained every 4–8 weeks after treatment. The tumour response was determined as a complete response (CR), partial response (PR), stable disease (SD), progressive disease, or not evaluated according to the Response Evaluation Criteria in Solid Tumors (Therasse *et al*, 2000). The best overall response for each patient was recorded as the tumour response. The response rate was calculated as CR + PR/all evaluated patients. Disease control was defined as CR, PR, or SD. The disease control rate was calculated as CR + PR + SD/all evaluated patients.

Pro-inflammatory cytokine assays. The serum levels of cytokines were measured using multiplex assays manufactured by Meso Scale Discovery (Gaithersburg, MD, USA). On the bottom of each well of 96-well plate-based assays, antibodies for GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p40 (IL-12), and TNF-α were spotted by the manufacturer. Following the capture of the cytokines by the spotted antibodies, label detection antibodies were bound to the antigen. The detection antibodies were coupled to electrochemiluminescent labels that emitted light when electrochemically stimulated via carbon-coated electrodes located in the bottom of the array wells. The resulting signal was read using a charge-coupled device. The MSD Multi-Spot Array assay was performed according to the manufacturer's instructions. The raw data were computed as the levels of electrochemiluminescent signals (light) measured using photodetectors and were analysed using Discovery Workbench 3.0 software (Meso Scale Discovery). A four-parameter logistic fit curve was generated for each analyte using the standards and the calculated concentration of each sample.

Statistical analyses. Progression-free survival (PFS) was defined as the time between the start of chemotherapy and either documented disease progression or death. Overall survival (OS) was defined as the interval between the initial administration of chemotherapy and either death or the last follow-up examination. Survival differences in the univariate analyses were calculated using the Cox's proportional hazards regression model. Factors that were strongly associated with a short survival period ($P<0.01$) were evaluated using a multivariate analysis of the Cox's proportional hazards regression model. Survival curves were drawn using the Kaplan–Meier method, and the difference between two survival curves was evaluated using the log-rank test. The frequency of patients in the two groups was compared using the Fisher's exact test. A comparison of non-categorical data was performed using the Mann–Whitney *U* test. The significance level was set at $P<0.05$. All the analyses were performed using the JMP 8 software, Windows version (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics. Between 2008 and 2009, 110 patients were enrolled in the study. Six patients were excluded from the study analysis because of the presence of inflammation at the start of chemotherapy, as follows: cholecystitis in three patients, cholangitis in two patients, and thrombophlebitis in both lower extremities in one patient. Four patients with rapid systemic weakness because of tumour progression refused to participate in the data collection after registering in the study. One patient with massive ascites who required multiple large-volume paracentesis procedures was judged unable to undergo systemic chemotherapy and was not evaluated in this study. Sixteen patients receiving S-1 monotherapy and 23 patients receiving GEM doublets were excluded because our focus was on the relationship between cytokine levels and the efficacy of GEM. The GEM doublets regimens consisted of GEM plus S-1 in 12 patients, GEM plus a cancer vaccine in 6 patients, and GEM plus axitinib in 5 patients. The remaining 60 patients were treated with GEM alone and were analysed in this study. The starting dose of GEM was 1000 mg m^{-2} in all the 60 patients. Patient characteristics and the clinical data obtained before chemotherapy are summarised in Table 1.

Pro-inflammatory cytokine levels. Each cytokine was studied in the following numbers of patients: GM-CSF ($n=58$), IFN-γ ($n=60$), IL-1β ($n=60$), IL-2 ($n=60$), IL-6 ($n=60$), IL-8 ($n=60$), IL-10 ($n=60$), IL-12 ($n=59$), and TNF-α ($n=60$) (Supplementary Table S1). The number of patients from whom samples were assayed was dependent on the accuracy of the measurement using the diluted sample. The following rates of detectable concentrations were observed: GM-CSF (33.5%), IFN-γ (20.0%), IL-1β (33.4%), IL-2 (20.0%), IL-6 (96.7%), IL-8 (100%), IL-10 (88.3%), IL-12 (37.3%), and TNF-α (98.3%). Undetectable concentrations of any cytokine were recorded as zero. According to the median value of each cytokine in all patients (Table 1), patients with higher concentrations than the median value were defined as the high cytokine group.

Tumour response and survival in patients with GEM alone. The tumour response was evaluated in all the 60 patients. None of the patients (0%) achieved a CR, and two patients (3.3%) had a PR. Twenty-nine patients (48.3%) were characterised as having SD, and one patient was categorised as not evaluated. The disease control rate was 51.6%. One patient was able to receive a pancreaticoduodenectomy after tumour reduction because of a good chemotherapeutic effect. The radiological and symptomatic progression of PC

Table 1. Patient characteristics

Variables		N (%)
Patients		60 (100)
Age (years)	Median (range)	66 (35–85)
Sex	Female	32 (53)
ECOG PS	0	32 (53)
	1	26 (43)
	2 or 3	2 (4)
Biliary drainage	Present	13 (22)
Opioid	Present	19 (32)
UICC-Stage	III	22 (37)
	IV	38 (63)
Liver metastasis	Present	29 (48)
Ascites	Present	21 (35)
Primary site	Head	19 (32)
Size of primary tumour (cm)	Median (range)	3.8 (1.8–9.7)
Second-line therapy	Chemotherapy	21 (35)
	Surgery	1 (2)
	Best supportive care	38 (63)
C-reactive protein (mg dl^{-1}dl)	Median (range)	0.36 (0.01–25.0)
GM-CSF (pg ml^{-1})	Median (range)	0.00 (0.00–289)
IFN-γ (pg ml^{-1})	Median (range)	0.00 (0.00–16.1)
IL-1β (pg ml^{-1})	Median (range)	0.00 (0.00–1.65)
IL-2 (pg ml^{-1})	Median (range)	0.00 (0.00–26.7)
IL-6 (pg ml^{-1})	Median (range)	1.93 (0.00–34.3)
IL-8 (pg ml^{-1})	Median (range)	19.6 (2.31–206)
IL-10 (pg ml^{-1})	Median (range)	1.81 (0.00–383)
IL-12 (pg ml^{-1})	Median (range)	0.00 (0.00–1700)
TNF-α (pg ml^{-1})	Median (range)	7.69 (0.00–23.0)

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group Performance Status; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor; UICC-Stage = stage based on the seventh criteria of the International Union Against Cancer (UICC).

were observed in 48 (80.0%) and 11 patients (18.4%), respectively. Twenty-one patients (35.0%) received second-line chemotherapy for advanced PC: S-1 ($n=18$) and S-1 + oxaliplatin ($n=2$). Fifty-four patients died from PC before the end of the observation period (August 2011). The median times for OS and PFS were 228 days (95% confidence interval (CI), 138–299 days) and 91 days (95% CI, 49–102 days), respectively.

Univariate and multivariate analyses for OS and PFS using serum levels of cytokines. The univariate and multivariate analysis for OS identified high IL-1β (HR 1.88; $P=0.048$) and high IL-6 (HR 2.10, $P=0.011$) levels as independent predictors of a poor OS (Table 2). In the univariate and multivariate analysis for PFS, a high IL-6 level was an independent risk factor for a short PFS (HR 2.32, $P=0.003$), and a high IL-1β level tended to be an independent risk factor for a poor PFS (HR 1.81, $P=0.056$).

To obtain detailed information regarding the efficacy of chemotherapy and the patient's prognosis according to the IL-6 and IL-1β concentrations, we tested the prognostic values of classifications based on the serum levels of IL-6 and IL-1β using survival curves of OS and PFS as follows: IL-6^{Low}/IL-1β^{Low} ($n=21$), IL-6^{Low}/IL-1β^{High} ($n=5$), IL-6^{High}/IL-1β^{Low} ($n=15$), and IL-6^{High}/IL-1β^{High} ($n=15$) (Figure 1). The OS and PFS curves of the IL-6^{High}/IL-1β^{High} group revealed higher risks for death and tumour progression than those of the IL-6^{Low}/IL-1β^{Low} group ($P<0.001$ in OS and $P<0.001$ in PFS). The difference between the IL-6^{High}/IL-1β^{Low} and the IL-6^{Low}/IL-1β^{Low} groups was obvious for PFS ($P=0.013$) and tended to be present for OS ($P=0.053$).

Prognosis and disease control classified according to the IL-6 and IL-1β status in patients with GEM alone. To identify the prognostic values of the IL-6/IL-1β classification, we calculated the risk of death and progression according to the status of the IL-6 and IL-1β levels. The relative risk of death and progression to the IL-6^{Low}/IL-1β^{Low} group was increased in the IL-6^{High}/IL-1β^{High} group (HR 4.06; $P<0.001$, HR 4.26; $P<0.001$) and in the IL-6^{High}/IL-1β^{Low} group (HR 1.90; $P=0.074$, HR 2.24; $P=0.021$) but not in the IL-6^{Low}/IL-1β^{High} group (HR 1.48; $P=0.497$, HR 1.68; $P=0.323$; Table 3).

Tumour control rates (TCRs) according to the IL-6 and IL-1β classifications were evaluated and are shown in Table 4. The TCRs of the IL-6^{High}/IL-1β^{High} and the IL-6^{High}/IL-1β^{Low} groups (20.0% and 40.0%) were lower than that of the IL-6^{Low}/IL-1β^{Low} group (76.0%, $P<0.001$ and $P=0.042$). A significant difference in the TCR between the IL-6^{High}/IL-1β^{High} group and the IL-6^{High}/IL-1β^{Low} group was not identified, but the actual value of TCR in the IL-6^{High}/IL-1β^{High} group was half of that in the IL-6^{High}/IL-1β^{Low} group.

GEM exposure according to IL-1β and IL-6 status. The median value of GEM dose intensity within 90 days after the start of chemotherapy (GEM-DI) was 737 mg m^{-2} per week in patients with GEM alone. GEM-DI was compared among the groups assigned the IL-6/IL-1β classification (Supplementary Table S2). The GEM-DI medians were increased in the IL-6^{High}/IL-1β^{High} (814 mg m^{-2} per week, $P=0.003$) and the IL-6^{High}/IL-1β^{Low} (781 mg m^{-2} per week, $P=0.012$) groups compared with the IL-6^{Low}/IL-1β^{Low} group (698 mg m^{-2} per week).

CRP levels according to IL-1β and IL-6 status. IL-6 and IL-1β promote the synthesis of CRP from hepatocyte (Morrone *et al*, 1988; Young *et al*, 2008). The serum CRP level is considered to be a good index for the physiological effects of IL-6 and IL-1β. We compared the CRP levels among the groups assigned to the IL-6/IL-1β classifications. The CRP level of the IL-6^{High}/IL-1β^{High} group was the highest of the groups with IL-6/IL-1β classifications (Table 5). The IL-6^{High}/IL-1β^{Low} group showed a higher CRP level than the IL-6^{Low}/IL-1β^{Low} group ($P=0.001$).

DISCUSSION

IL-6 is a pleiotropic cytokine with a variety of effects on cells and tissues (Trikha *et al*, 2003) that is synthesised by many different cell types, including immune cells, fibroblasts, endothelial cells, myocytes, adipocytes, a variety of endocrine cells, and PC cells (Tracey and Cerami, 1993; Van Snick, 1996; Fried *et al*, 1998; Martignoni *et al*, 2005). IL-6 mRNA is found in 64% of PC cases, in which the IL-6 mRNA expression ratio in relation to normal pancreatic tissue is strongly upregulated by a median of 62.4-fold (Bellone *et al*, 2006). The immunohistochemical expression of IL-6 in PC tumours is strong in the cytoplasm of PC cells and weak in inflammatory cells (Martignoni *et al*, 2005). Furthermore, the serum IL-6 level in patients with PC is higher than in healthy individuals (Okada *et al*, 1998; Barber *et al*, 1999; Ebrahimi *et al*, 2004; Martignoni *et al*, 2005; Talar-Wojnarowska *et al*, 2009). A high IL-6 level is correlated with tumour aggressiveness, inflammatory response, and systemic weakness, such as large tumour size, hepatic metastasis, an elevated level of serum CRP, body weight loss, and poorer performance status (Okada *et al*, 1998;

Table 2. Univariate and multivariate analyses for overall survival and progression-free survival according to cytokine level in patients receiving gemcitabine monotherapy for advanced pancreatic cancer

			Univariate analysis		Multivariate analysis	
Tested factor		**N**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Overall survival						
GM-CSF	High	20	1.84 (1.02–3.21)	0.042		
IFN-γ	High	12	1.16 (0.53–2.29)	0.686		
IL-1β	High	20	2.33 (1.27–4.18)	0.007	1.88 (1.01–3.45)	0.048
IL-2	High	12	2.09 (1.01–4.00)	0.048		
IL-6	High	30	2.41 (1.39–4.20)	0.002	2.10 (1.19–3.74)	0.011
IL-8	High	29	1.49 (0.87–2.57)	0.149		
IL-10	High	30	1.22 (0.71–2.11)	0.465		
IL-12	High	22	2.06 (1.12–3.72)	0.020		
TNF-α	High	30	0.98 (0.57–1.68)	0.939		
Progression-free survival						
GM-CSF	High	20	1.61 (0.91–2.76)	0.098		
IFN-γ	High	12	1.27 (0.64–2.33)	0.481		
IL-1β	High	20	2.33 (1.30–4.08)	0.005	1.81 (0.98–3.27)	0.056
IL-2	High	12	2.08 (1.02–3.97)	0.043		
IL-6	High	30	2.67 (1.56–4.56)	<0.001	2.32 (1.33–4.07)	0.003
IL-8	High	29	1.27 (0.75–2.14)	0.362		
IL-10	High	30	1.46 (0.87–2.45)	0.148		
IL-12	High	22	2.13 (1.21–3.72)	0.010		
TNF-α	High	30	1.15 (0.68–1.93)	0.595		

Abbreviations: CI = confidence interval; GM-CSF = granulocyte macrophage colony-stimulating factor; HR = hazard ratio; IFN = interferon; IL = interleukin; TNF = tumour necrosis factor.

Barber *et al*, 1999; Ebrahimi *et al*, 2004; Martignoni *et al*, 2005; Talar-Wojnarowska *et al*, 2009). The prognostic impact of the circulating IL-6 level was demonstrated in a study by Ebrahimi *et al* (2004), in which patients underwent either pancreatic resection or chemotherapy. This study clearly highlights the independent prognostic value of a high IL-6 level on OS in patients receiving GEM for PC. The correlation between high IL-6 levels and a shortened PFS was observed in hepatocellular carcinoma patients receiving sunitinib monotherapy (Zhu *et al*, 2009) and in diffuse large-cell lymphoma patients receiving chemotherapy (Seymour *et al*, 1995). To the best of our knowledge, the association between serum IL-6 levels and PFS in patients undergoing systemic chemotherapy for PC has not been previously reported. This study clearly showed the impact of a high IL-6 level on a shortened PFS in patients undergoing GEM for PC.

IL-1β is a pro-inflammatory cytokine that is synthesised by many cell types, including monocytes, tissue macrophages, and PC cells (Bellone *et al*, 2006; Angst *et al*, 2008). IL-1β mRNA can be identified in >80% of PC tumour tissues, and the IL-1β mRNA expression ratio in relation to normal pancreatic tissue in resected PC specimens is, on average, strongly upregulated by 28.5-fold (Ebrahimi *et al*, 2004; Bellone *et al*, 2006). IL-1β from tumour cells and monocytes contributes to the chemoresistance of PC cells (Arlt *et al*, 2002; Angst *et al*, 2008). The serum levels of IL-1β are rarely measured in healthy tissues. In fact, the total daily production of IL-1β was calculated to be approximately 6 ng day^{-1} in a study using a specific antibody to human IL-1β (Lachmann *et al*, 2009), whereas in humans injected with an endotoxin, the levels of IL-1β were below the detection limit (<2 pg ml^{-1}) at baseline and were elevated for approximately 2 h, reaching maximal concentrations of 50–60 pg ml^{-1} (Granowitz *et al*, 1991). No relationship has been reported between the serum IL-1β level and its clinical significance in PC patients because the serum IL-1β levels are usually below the lower measurable limit of detection (LOD). The LOD for IL-1β was previously found to be 0.3 pg ml^{-1} using an enzyme-linked immunosorbent assay (Ebrahimi *et al*, 2004). In this study, the LOD of IL-1β was 0.19 pg ml^{-1} ml^{-1}, and the detectable rate of serum IL-1β was 33.4%. Our assay for the detection of pro-inflammatory cytokines was based on electrochemiluminescence, which is a superior detection method compared with enzyme-linked immunosorbent assay; hence, our LOD was lower. Recent progress in assay methods has improved the detection of serum IL-1β, enabling the use of the serum IL-1β concentration for predicting the efficacy of chemotherapy and the identification of a patient's prognosis in this study. A high IL-1β serum level was an independent prognostic factor that, in this study, showed a tendency toward an association with a shortened PFS. IL-1β promotes metastasis and angiogenesis because of the upregulation of pro-metastatic genes and molecules, including matrix metalloproteinases and endothelial adhesion molecules, along with vascular endothelial cell growth factor, chemokines, growth factors, and TGFβ (Dinarello, 2010). A high IL-1β level may be related to an aggressive tumour status and may be correlated with a poor prognosis.

The IL-6^{High}/IL-1β^{High} group had shortened PFS and OS compared with the IL-6^{Low}/IL-1β^{Low} group. The disease control rate in the IL-6^{High}/IL-1β^{High} group was decreased by one-fourth compared with that of the IL-6^{Low}/IL-1β^{Low} group. Interestingly, GEM-DI in the IL-6^{High}/IL-1β^{High} was higher than in the IL-6^{Low}/IL-1β^{Low} group. The CRP serum level, a good index of the IL-6 and IL-1β effects via STAT3 and NF-κB, was higher in the IL-6^{High}/IL-1β^{High} group. These results may indicate that the resistance of PC tumour cells against GEM was dependent on the effects of IL-6 and IL-1β via STAT3 and NF-κB. GEM leads to DNA damage in PC cells, which results in GEM-induced apoptosis (Arlt *et al*, 2010). The resistance of PC cells to chemotherapeutic agents is due to an altered balance between pro- and anti-apoptotic proteins, resulting in reduced apoptotic responsiveness

(Grivennikov and Karin, 2010). Bcl-2 and Bcl-xL are anti-apoptotic proteins that are activated by STAT3 and NF-κB, whereas Mcl-1, another of the anti-apoptotic proteins, is primarily STAT3-dependent (Arlt *et al*, 2010). IL-6 and IL-1β can activate STAT3 and NF-κB (Nishikawa *et al*, 2008), possibly resulting in an increase of anti-apoptotic proteins in PC cells. Based on the above context, the inhibition of STAT3 and NF-κB was expected to resolve the chemoresistance of PC cells.

The IL-6^{High}/IL-$1\beta^{Low}$ group had poor outcomes for OS and PFS compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. The disease control rate in the IL-6^{High}/IL-$1\beta^{Low}$ group was reduced to half of that in the IL-6^{Low}/IL-$1\beta^{Low}$ group, though GEM-DI in the IL-6^{High}/IL-$1\beta^{Low}$ was higher than in the IL-6^{Low}/IL-$1\beta^{Low}$ group. CRP was able to be synthesised by the effect of IL-6 alone, and the CRP concentration was elevated in the IL-6^{High}/IL-$1\beta^{Low}$ group compared with the IL-6^{Low}/IL-$1\beta^{Low}$ group. These results imply that the PC tumour cells were resistant to GEM via IL-6 only.

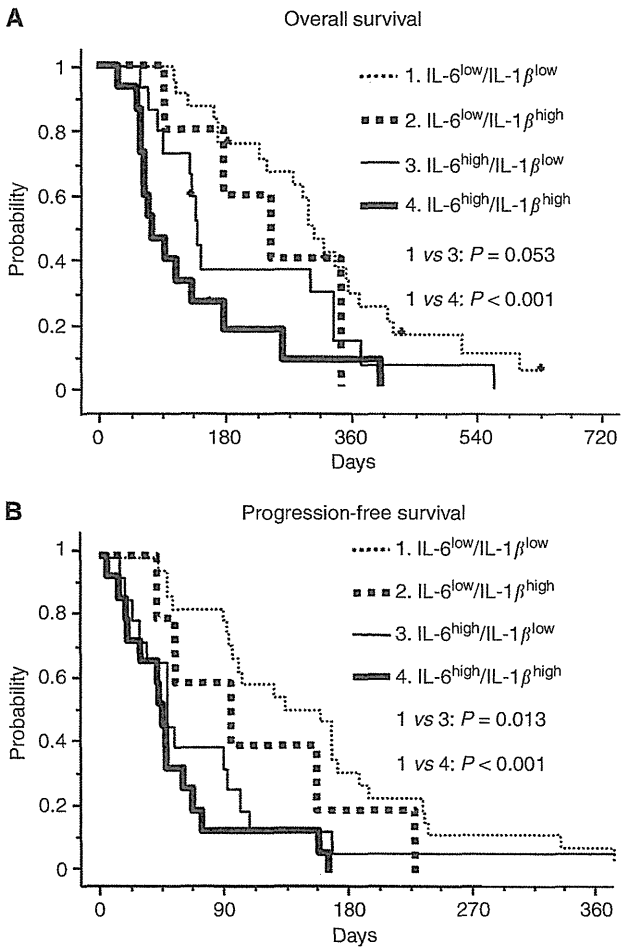

Figure 1. The OS and PFS curves according to the status of IL-6 and IL-1β. (**A**) OS and (**B**) PFS curves in the IL-6^{Low}/IL-$1\beta^{Low}$ (dotted line), the IL-6^{Low}/IL-$1\beta^{High}$ (bold dotted line), the IL-6^{High}/IL-$1\beta^{Low}$ (solid line), and the IL-6^{High}/IL-$1\beta^{High}$ groups (bold line).

Table 4. Tumour control rates according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (%)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	76.0 (56.6–88.5)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	60.0 (23.1–88.2)	0.589
IL-6^{High}/IL-$1\beta^{Low}$	15	40.0 (19.8–64.3)	0.042
IL-6^{High}/IL-$1\beta^{High}$	15	20.0 (7.0–45.2)	<0.001

Abbreviations: CI = confidence interval; IL = interleukin.

Table 5. CRP level according to serum levels of IL-6 and IL-1β in patients with gemcitabine monotherapy for advanced pancreatic cancer

IL-6/IL-1β classification	N	Median (95% CI) (mg dl^{-1})	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	0.13 (0.06–0.25)	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	0.08 (NA)	0.140
IL-6^{High}/IL-$1\beta^{Low}$	15	1.19 (0.17–2.79)	0.001
IL-6^{High}/IL-$1\beta^{High}$	15	5.61 (2.83–10.09)	<0.001

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; IL = interleukin; NA = not applicable; OS = overall survival; PFS = progression-free survival.

Table 3. Impacts of the classification using IL-6 and IL-1β levels on overall survival and progression-free survival in patients with gemcitabine monotherapy for advanced pancreatic cancer

Overall survival

IL-6/IL-1β classification	N	Median OS (95%CI) (days)	HR (95% CI)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	306 (228–355)	1	Ref.
IL-6^{Low}/IL-$1\beta^{High}$	5	246 (97–346)	1.48 (0.43–3.97)	0.497
IL-6^{High}/IL-$1\beta^{Low}$	15	140 (83–334)	1.90 (0.94–3.72)	0.074
IL-6^{High}/IL-$1\beta^{High}$	15	79 (61–134)	4.06 (1.96–8.18)	<0.001

Progression-free survival

IL-6/IL-1β classification	N	Median PFS (95%CI) (days)	HR (95% CI)	*P*-value
IL-6^{Low}/IL-$1\beta^{Low}$	25	158 (96–187)	1	ref
IL-6^{Low}/IL-$1\beta^{High}$	5	96 (42–229)	1.68 (0.56–4.11)	0.323
IL-6^{High}/IL-$1\beta^{Low}$	15	48 (23–92)	2.24 (1.14–4.29)	0.021
IL-6^{High}/IL-$1\beta^{High}$	15	46 (19–61)	4.26 (2.08–8.55)	<0.001

Abbreviations: CI = confidence interval; HR = hazard ratio; IL = interleukin; OS = overall survival; PFS = progression-free survival.

IL-6 binds a nonsignalling α-receptor (IL-6 receptor), and the dimerisation of gp130 (a signalling β-receptor) and the binding of IL-6 to its receptor lead to the activation of receptor-associated kinases within the cell. These lead to the phosphorylation of proximal tyrosine residues within the intracellular portion of gp130 and the subsequent control of STAT1 and STAT3 activity (Jones *et al*, 2011). Inhibition of the above IL-6 pathway would improve the resistance against GEM in PC tumour cells.

In conclusion, this study demonstrated that the serum levels of IL-6 and IL-1β were predictive of both the efficacy of GEM and the prognosis of patients with advanced PC. Inhibition of the IL-6 and IL-1β pathways may be a candidate target for novel therapies for advanced PC.

ACKNOWLEDGEMENTS

We thank Ms Kayo Takei, Ms Miho Akimoto, Ms Yuriko Sato, and Ms Youko Yamasaki of the National Cancer Center Hospital East for their secretarial support and Mr Ryuu Kasai of Chugai Pharmaceutical Co., Ltd. for support with the statistical analysis. This work was supported by Grants-in-aid for Cancer Research and for the Third-term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan; JSPS KAKENHI Grant Number 22790624; the National Cancer Center Research and Development Fund (23-A-2b); and Chugai Pharmacology Co. Ltd.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aggarwal BB, Vijayalekshmi RV, Sung B (2009) Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin Cancer Res* **15**: 425–430.

Angst E, Reber HA, Hines OJ, Eibl G (2008) Mononuclear cell-derived interleukin-1beta confers chemoresistance in pancreatic cancer cells by upregulation of cyclooxygenase-2. *Surgery* **144**: 57–65.

Arlt A, Müerköster SS, Schäfer H (2010) Targeting apoptosis pathways in pancreatic cancer. *Cancer Letter;* e-pub ahead of print 13 November 2010.

Arlt A, Vorndamm J, Müerköster S, Yu H, Schmidt WE, Fölsch UR, Schäfer H (2002) Autocrine production of interleukin 1beta confers constitutive nuclear factor kappaB activity and chemoresistance in pancreatic carcinoma cell lines. *Cancer Res* **62**: 910–916.

Barber MD, Fearon KC, Ross JA (1999) Relationship of serum levels of interleukin-6, soluble interleukin-6 receptor and tumour necrosis factor receptors to the acute-phase protein response in advanced pancreatic cancer. *Clin Sci (Lond)* **96**: 83–87.

Bellone G, Smirne C, Mauri FA, Tonel E, Carbone A, Buffolino A, Dughera L, Robecchi A, Pirisi M, Emanuelli G (2006) Cytokine expression profile in human pancreatic carcinoma cells and in surgical specimens: implications for survival. *Cancer Immunol Immunother* **55**: 684–698.

Dinarello CA (2010) Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev* **29**: 317–329.

Dorff TB, Goldman B, Pinski JK, Mack PC, Lara Jr PN, Van Veldhuizen Jr PJ, Quinn DI, Vogelzang NJ, Thompson Jr IM, Hussain MH (2010) Clinical and correlative results of SWOG S0354: a phase II trial of CNTO328 (siltuximab), a monoclonal antibody against interleukin-6, in chemotherapy-pretreated patients with castration-resistant prostate cancer. *Clin Cancer Res* **16**: 3028–3034.

Ebrahimi B, Tucker SL, Li D, Abbruzzese JL, Kurzrock R (2004) Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* **101**: 2727–2736.

Fried SK, Bunkin DA, Greenberg AS (1998) Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* **83**: 847–850.

Ganapathi MK, May LT, Schultz D, Brabenec A, Weinstein J, Sehgal PB, Kushner I (1998) Role of interleukin-6 in regulating synthesis of C-reactive protein and serum amyloid A in human hepatoma cell lines. *Biochem Biophys Res Commun* **157**: 271–277.

Granowitz EV, Santos AA, Poutsiaka DD, Cannon JG, Wilmore DW, Wolff SM, Dinarello CA (1991) Production of interleukin-1-receptor antagonist during experimental endotoxaemia. *Lancet* **338**: 1423–1424.

Grivennikov S, Karin M (2010) Dangerous liaisons: STAT3 and NF-kB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev* **21**: 11–19.

Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, McQuitty M, Hunter DS, Levy R, Knoops L, Cervantes F, Vannucchi AM, Barbui T, Barosi G (2012) JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N Engl J Med* **366**: 787–798.

Ioka T, Ikeda M, Ohkawa S, Yanagimoto H, Fukutomi A, Sugimori K, Baba H, Yamao K, Shimamura T, Chen J, Mizumoto K, Furuse J, Funakoshi A, Hatori T, Yamaguchi T, Egawa S, Sato A, Ohashi Y, Cheng A, Okusaka T (2011) 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* **29**, (suppl) abstr 4007.

Jones G, Sebba A, Gu J, Lowenstein MB, Calvo A, Gomez-Reino JJ, Siri DA, Tomsic M, Alecock E, Woodworth T, Genovese MC (2010) Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. *Ann Rheum Dis* **69**: 88–96.

Jones SA, Scheller J, Rose-John S (2011) Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J Clin Invest* **121**: 3375–3383.

Kindler HL, Ioka T, Richel DJ, Bennouna J, Létourneau R, Okusaka T, Funakoshi A, Furuse J, Park YS, Ohkawa S, Springett GM, Wasan HS, Trask PC, Bycott P, Ricart AD, Kim S, Van Cutsem E (2011) 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.

Kuemmerle-Deschner JB, Hachulla E, Cartwright R, Hawkins PN, Tran TA, Bader-Meunier B, Hoyer J, Gattorno M, Gul A, Smith J, Leslie KS, Jiménez S, Morell-Dubois S, Davis N, Patel N, Widmer A, Preiss R, Lachmann HJ (2011) Two-year results from an open-label, multicentre, phase III study evaluating the safety and efficacy of canakinumab in patients with cryopyrin-associated periodic syndrome across different severity phenotypes. *Ann Rheum Dis* **70**: 2095–2102.

Lachmann HJ, Lowe P, Felix SD, Rordorf C, Leslie K, Madhoo S, Wittkowski H, Bek S, Hartmann N, Bosset S, Hawkins PN, Jung T (2009) *In vivo* regulation of interleukin 1beta in patients with cryopyrin-associated periodic syndromes. *J Exp Med* **206**: 1029–1036.

Martignoni ME, Kunze P, Hildebrandt W, Künzli B, Berberat P, Giese T, Klöters O, Hammer J, Büchler MW, Giese NA, Friess H (2005) Role of mononuclear cells and inflammatory cytokines in pancreatic cancer-related cachexia. *Clin Cancer Res* **11**: 5802–5808.

Morizane C, Okusaka T, Morita S, Tanaka K, Ueno H, Kondo S, Ikeda M, Nakachi K, Mitsunaga S (2011) Construction and validation of a prognostic index for patients with metastatic pancreatic adenocarcinoma. *Pancreas* **40**: 415–421.

Morrone G, Ciliberto G, Oliviero S, Arcone R, Dente L, Content J, Cortese R (1988) Recombinant interleukin 6 regulates the transcriptional activation of a set of human acute phase genes. *J Biol Chem* **263**: 12554–12558.

Nishikawa T, Hagihara K, Serada S, Isobe T, Matsumura A, Song J, Tanaka T, Kawase I, Naka T, Yoshizaki K (2008) Transcriptional complex formation of c-Fos, STAT3, and hepatocyte NF-1 is essential for cytokine-driven C-reactive protein gene expression. *J Immunol* **180**: 3492–3501.

Okada S, Okusaka T, Ishii H, Kyogoku A, Yoshimori M, Kajimura N, Yamaguchi K, Kakizoe T (1998) Elevated serum interleukin-6 levels in patients with pancreatic cancer. *Jpn J Clin Oncol* **28**: 12–15.

Seymour JF, Talpaz M, Cabanillas F, Wetzler M, Kurzrock R (1995) Serum interleukin-6 levels correlate with prognosis in diffuse large-cell lymphoma. *J Clin Oncol* **13**: 275–282.

Sobin LH, Gospodarowicz MK, Wittekind C (2009) *TNM Classification of Malignant Tumors*. 7th edn (Wiley-Blackwell: Oxford, UK).

Talar-Wojnarowska R, Gasiorowska A, Smolarz B, Romanowicz-Makowska H, Kulig A, Malecka-Panas E (2009) Clinical significance of interleukin-6 (IL-6) gene polymorphism and IL-6 serum level in pancreatic adenocarcinoma and chronic pancreatitis. *Dig Dis Sci* **54**: 683–689.