Table 1 Clinicopathological profiles of the 31 patients

	mean ± SD (range) or *n* (%)
Age (yr)	65 ± 9 (44-82)
Gender (M:F)	22:9
Tumor location	
Head	17 (55)
Body	11 (35)
Tail	3 (10)
Maximum tumor diameter (cm)	3.8 ± 2.0 (1.3-11.0)
SUV	6.5 ± 3.3 (2.5-15.8)

SUV: Standardized uptake value.

Table 2 Correlations between tumor size and sensitivity of positron emission tomography, computed tomography, magnetic resonance imaging or tumor markers

TS (cm)	*n*	PET (%)	CT (%)	MRI (%)	CEA (%)	CA19-9 (%)
TS1 (≤ 2)	5	100[a]	40	0	0	40
TS2 (> 2, ≤ 4)	15	93	93	89	20	73
TS3-4 (> 4)	11	100	100	100	73	91

TS: Tumor size; PET: Positron emission tomography; CT: Computed tomography; MRI: Magnetic resonance imaging; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9. [a]$P = 0.002$ *vs* MRI or CEA, $P = 0.038$ *vs* CT or CA19-9.

the sensitivity of FDG-PET, CT, MRI, CEA and CA19-9. The Student *t* test was used to compare the values of the SUV between the groups. All statistical analyses were performed using SPSS software (SPSS, Chicago, USA). A P value < 0.05 was considered to be statistically significant.

RESULTS

Table 1 shows the clinicopathological profiles of the 31 patients. The sensitivity of FDG-PET, CT, MRI, the serum levels of CEA and CA19-9 were 100%, 40%, 0%, 0%, 40% in TS1, 93%, 93%, 89%, 20%, 73% in TS2 and 100%, 100%, 100%, 73%, 91% in TS3-4 (Table 2). The sensitivity of PET for detecting TS1, TS2, and TS3 tumors was 100%, 93%, and 100%, respectively. The sensitivity of FDG-PET was significantly higher in comparison to CT, MRI and the serum levels of CEA and CA19-9 in the patients with TS1 ($P = 0.002$ *vs* MRI or CEA, $P = 0.038$ *vs* CT or CA19-9).

Although the sensitivity was higher for larger tumors, the SUV was not significantly associated with the TS factor. The mean SUV did not show a significant difference in relation to the TS (TS1: 5.8 ± 4.5, TS2: 5.7 ± 2.2, TS3-4: 8.2 ± 3.9), respectively. The diagnosis of pancreatic adenocarcinoma was histologically confirmed in all patients with TS1 cancer (Table 3). The tumor was well differentiated in 4 patients and poorly differentiated in one patient. The tumor diameter ranged from 13 to 20 mm. All the TS1 tumors showed higher SUVs in the delayed phase compared with that in the early phase. The SUV pattern suggested the small lesions were malignant tumors.

Table 3 Characteristics of TS1 pancreas cancer

	Age (yr)	Gender	Size (mm)	Tumor differentiation	SUV early	SUV delayed
1	77	F	13	Poor	3.59	4.16
2	77	M	20	Well	5.53	7.10
3	82	F	20	Well	2.74	3.14
4	68	M	18	Well	2.87	3.06
5	81	M	20	Well	12.79	13.78

Poor: Poorly differentiated adenocarcinoma; Well: Well differentiated adenocarcinoma; SUV: Standardized uptake value; SUV early: Value at 1 h after iv ^{18}F-fluorodeoxyglucose; SUV delayed: Value at 2 h.

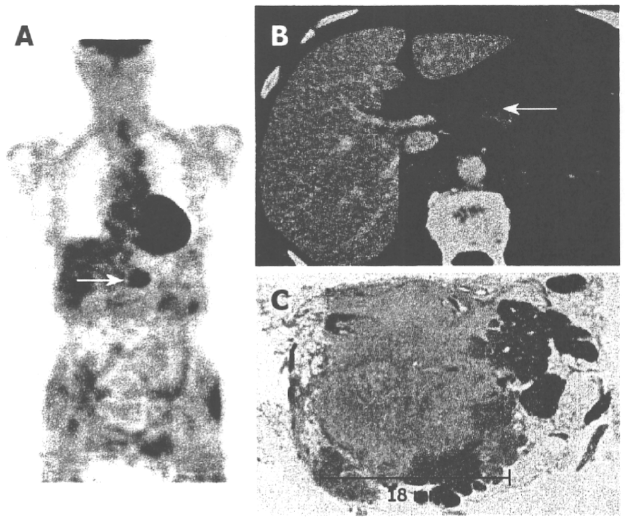

Figure 1 Positron emission tomography images of a 68-year-old male with TS1 pancreatic cancer. A: Whole body positron emission tomography image shows apparent increased uptake of ^{18}F-fluorodeoxyglucose in the tumor (arrow, delayed point standardized uptake value, 3.06); B: Axial computed tomography image with contrast enhancement shows small low-density mass in the pancreas body (arrow); C: The histological findings (HE staining) of the pancreas revealed invasive ductal cancer in the body of the pancreas with a diameter of 18 mm.

Representative images of one patient (case 4 in Table 3) with TS1 pancreas cancer are shown in Figure 1. A 68-year-old male was transferred to our hospital for evaluation and further management of diabetes mellitus. A whole body FDG-PET image shows apparent increased uptake in the tumor (delayed point SUV, 3.06) (Figure 1A). An axial CT image with contrast enhancement shows a small low-density mass in the pancreas body (Figure 1B). The histological findings (HE staining) of the pancreas revealed invasive ductal cancer in the body of pancreas with a diameter of 18 mm (Figure 1C).

DISCUSSION

The usefulness of FDG-PET in diagnosing distant disease from advanced pancreatic cancer has been previously reported, although the poor spatial resolution of FDG-PET is known to limit the local staging of pancreatic cancer[3]. CT is better suited to demonstrate the relationship of the tumor, adjacent organs, and vascular structure in advanced pancreatic cancer, but it is rela-

tively insensitive for detecting pancreatic cancers < 2 cm in size[8-11]. Although the sensitivity of contrast-enhanced helical CT in the detection of pancreatic carcinoma is reported to vary from 76% to 92%, the sensitivity declines to 58% to 67% for tumors smaller than 2 cm[8-10,12]. The sensitivity of EUS or MRI has been reported to be the same or slightly better in comparison to that of CT[13,14].

Patients with small pancreatic carcinoma have no typical symptoms, which make it very difficult to detect. In contrast to the inherent limitations of this anatomic imaging modality, functional imaging using FDG PET appears to represent a significant advance in the detection of small pancreatic cancers < 2 cm in size. Seo *et al*[15] reported the effectiveness of FDG-PET for the detection of small pancreatic cancers with a sensitivity of 81% for tumors smaller than 2 cm. Although there have been a few reports indicating the value of FDG-PET in the diagnosis of small pancreatic cancer, the efficacy of dual phase FDG-PET in small pancreatic cancer has not been fully evaluated.

Dual time point FDG-PET is a more reliable method than single time point FDG-PET for differentiating pancreatic cancer from a mass identified to be chronic pancreatitis. In addition, delayed PET imaging is also helpful for identifying more lesions in patients with pancreatic cancer[7]. Dual time point evaluation is routinely performed in our institution for patients with pancreatic cancer. There were 5 tumors smaller than 2 cm in the current series, and the sensitivity of FDG-PET for the detection of these tumors was 100%, although there was no tumor smaller than 1 cm. A dual time point evaluation may help to increase the sensitivity in the diagnosis of small pancreatic cancer.

The increased uptake of FDG due to the enhanced glucose metabolism of cancer cells is a sensitive marker of tumor viability or biological behavior. The SUV is an independent prognostic factor in various malignant tumors. Sperti *et al*[16] demonstrated that a high SUV (> 4.0) was associated with shorter survival. Maemura *et al*[17] reported that pancreatic tumors with distant metastases showed significantly higher SUV levels than tumors without metastases. The present study showed the SUVs of pancreatic cancer did not differ significantly in relation to tumor size. The results indicate that FDG-PET may, therefore be useful even in patients with small pancreatic cancers that can not be visualized by either CT or other modalities. The present study did not provide data on the specificity because there were no benign lesions. In our previous study[7], the specificity of FDG-PET for detection of pancreatic cancer was 65%. Benign lesions such as chronic pancreatitis and autoimmune-related pancreatitis can also accumulate FDG and result in false-positive interpretations of PET studies. Further studies including benign lesions are required to clarify the diagnostic accuracy of FDG-PET.

The routine use of PET is not believed to be cost-effective and thus has not been accepted as a standard screening examination for small pancreatic cancer. Although the etiology of pancreatic cancer has not yet been completely elucidated, several factors are thought to be associated with cancer[18-21]. Smoking is a consistently identified environmental risk factor which doubles the risk of pancreatic cancer[19,20]. Dietary factors, such as high energy intake, cholesterol, and high meat consumption are known to increase the risk. Long-standing diabetes, chronic pancreatitis and certain hereditary conditions can affect the risk of developing pancreatic cancer. FDG-PET screening is therefore recommended if the patients are elderly and have been identified to be at risk for pancreatic cancer. FDG-PET screening for the detection of pancreatic cancers should therefore be considered for patients with chronic pancreatitis, because such patients are 16 times more likely to develop pancreatic cancer than healthy controls. Dual time point FDG-PET is a reliable method for differentiating pancreatic cancer from a mass identified to be chronic pancreatitis[22]. However, there is a limitation in our study. This study was performed by a PET scanner. The coregistration of CT and PET images or integrated PET/CT devices may help to improve some diagnostic problems. Further evolution of PET scanner technology, including the PET/CT hybrid scanner, should provide superior diagnostic performance.

These results indicate that FDG-PET is a useful modality for the detection of small pancreatic cancers with a diameter of less than 20 mm. However, this study was limitated due to the small population of patients. As a result, further prospective studies with PET/CT involving a larger population of patients should therefore be conducted to substantiate the results of this study.

COMMENTS

Background

Early diagnosis is the most important factor for improving the overall survival and quality of life in patients with pancreatic cancer. Positron emission tomography (PET) has demonstrated superiority to computed tomography (CT), ultrasonography (US), and endoscopic US (EUS) in its sensitivity and specificity in diagnosing pancreas cancer.

Research frontiers

Delayed additional ^{18}F-fluorodeoxyglucose PET (FDG-PET) imaging is a useful method in differential diagnosis of malignant from benign lesions. However, the role of dual time point FDG-PET in the diagnosis of small pancreatic cancers has yet to be established.

Innovations and breakthroughs

The usefulness of FDG-PET in diagnosing distant disease from advanced pancreatic cancer has previously been reported, although the poor spatial resolution of FDG-PET is known to limit the local staging of pancreatic cancer. This is the first study to describe the usefulness of dual time point FDG-PET in detection of small pancreatic cancers with a diameter of less than 20 mm.

Applications

The ability to diagnose the early stage of pancreas cancer can be improved by using the dual time point FDG-PET in combination with CT, US and EUS. Early diagnosis is the most important factor for improving the overall survival and quality of life in patients with pancreatic cancer.

Terminology

Dual time point FDG-PET: FDG, a glucose analog, is taken up by high-glucose-using cells such as brain, kidney, and cancer cells, where phosphorylation prevents the glucose from being released intact. FDG-PET can be used for diagnosis, staging, and monitoring treatment of cancers. PET scans detect the areas with increased glucose uptake. The standardized uptake value of FDG is measured from two sequential time points.

Peer review

This article is a retrospective analysis concerning a diagnostic value of PET for small pancreatic cancer. It is well-written but there are several issues to be resolved.

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Association of aldehyde dehydrogenase 2 gene polymorphism with pancreatic cancer but not colon cancer

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Aims: Most of the acetaldehyde, a recognized animal carcinogen, generated during alcohol metabolism is eliminated by liver mitochondrial aldehyde dehydrogenase 2 (ALDH2). More than 40% of Japanese people have the inactive form of ALDH2, and inactive ALDH2 is a risk factor for multiple cancer of the esophagus, as well as head and neck cancer. Possible associations between pancreatic cancer and *ALDH2* gene polymorphism, as well as between colon cancer and *ALDH2* gene polymorphism, in conjunction with smoking and/or drinking habits, were examined in a Japanese population.

Methods: Patients with pancreatic cancer ($n = 187$) and with colon cancer ($n = 49$) were examined. The drinking (5 g ethanol consumption/day) and/or smoking habits as well as *ALDH2* gene polymorphism were examined. The age-matched control subjects were recruited in the NILS Longitudinal Study of Aging (LSA).

Results: Aging, smoking and inactive ALDH2, but not alcohol, are independent risk factors for pancreatic cancer. The frequency of smoking habits tended to be higher in patients with colon cancer compared with the patients without cancer. However, age, body mass index or the distribution of *ALDH2* genotypes did not differ significantly among the patients with colon cancer, colon polyps and others.

Conclusions: Inactive ALDH2 is an independent risk factor for pancreatic cancer, but inactive ALDH2 might not be a risk for colon cancer. **Geriatr Gerontol Int 2010; 10 (Suppl. 1): S120–S126.**

Keywords: alcohol, ALDH2, colon cancer, pancreatic cancer, smoking.

Introduction

In 2005, people over 65 years of age made up more than 20% of the Japanese popul[illegible]. Lifestyle related diseases, such as malignant neoplasms, cardiovascular diseases and cerebrovascular diseases, are becoming more prevalent as a cause of death. At present, malignant neoplasms are the most common cause of death (http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suikei05/index.html). Colon cancer, lung cancer, and pancreatic cancer are increasing in [illegible] men and women (Figs 1 and 2). The International agency for Research on Cancer (IARC) Monographs (http://monographs.iarc.fr/) identify environmental factors that can increase the risk of human cancer. These include chemicals, complex mixtures, occupational exposure, physical and biological agents, and lifestyle factors. Since 1971, more than 900 agents have been evaluated, of which approximately 400 have been identified as carcinogenic or potentially carcinogenic to humans.

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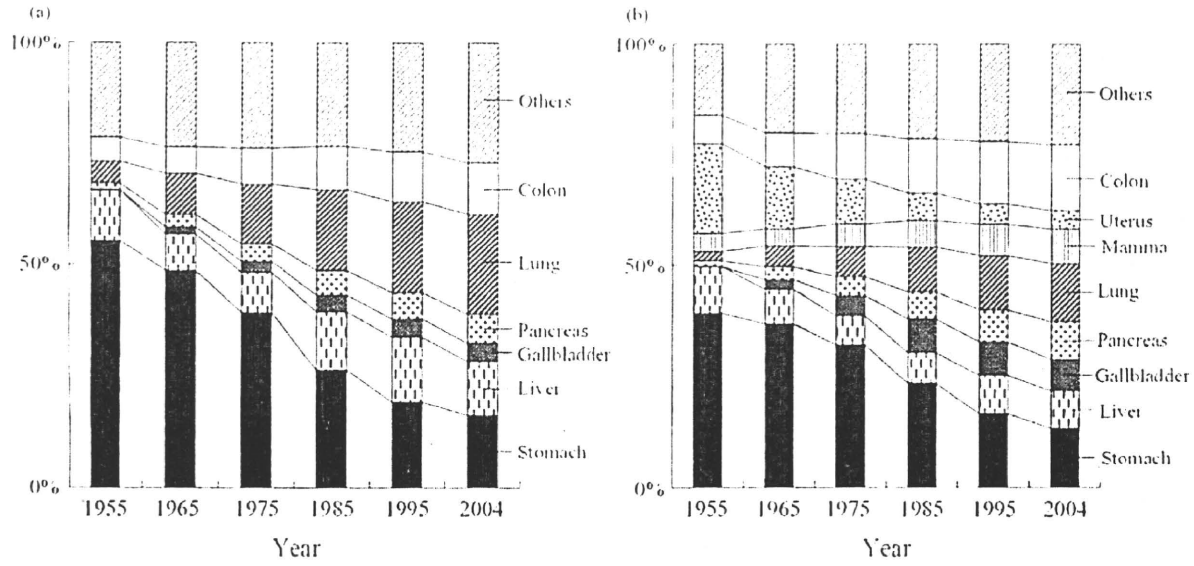

Figure 1 Changes in cause of death by malignant neoplasm since 1955. Data are derived from: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suii05/deth15.html. (a) Male; (b) Female.

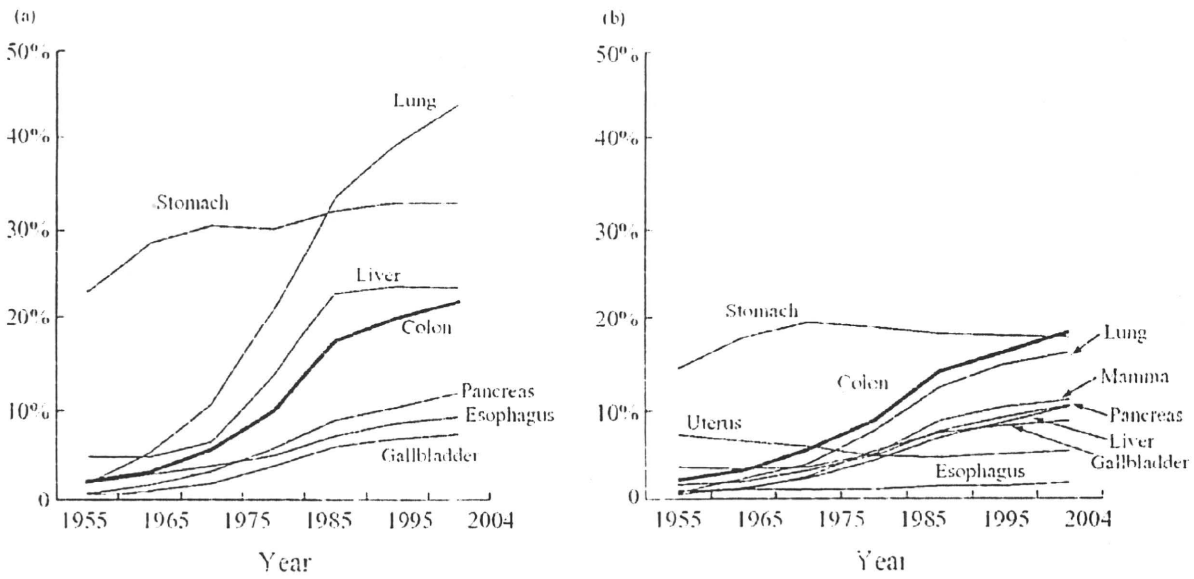

Figure 2 Percent changes in cause of death by malignant neoplasm. Data are derived from: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suii05/deth16.html. (a) Male; (b) Female.

A smoking habit has been known to be a risk factor for various cancers, such as lung and pancreatic cancer.[1] Moderate alcohol consumption has some health benefits, such as increasing high-density lipoprotein (HDL) and decreasing stress. However, the WHO identified the consumption of alcohol as one of the top 10 risks for the worldwide burden of disease.[2] Many studies have consistently shown that regular alcohol consumption is associated with an increased risk for cancers of the oral cavity, pharynx, larynx and esophagus (Table 1).[3–5]

The Japanese population is deficient in aldehyde dehydrogenase (ALDH2) because of the high frequency of mutant alleles in the *ALDH2* gene (*ALDH2**2). The *ALDH2**2 allele encodes a Glu-to-Lys amino acid

Table 1 Alcohol consumption and cancer

Oral cavity
Pharynx
Larynx
Esophagus: *ALDH2*1/2*2*
Colon, rectum
Breast (female)

Data are reproduced from reference 12.

substitution at the 14th and last codon. More than 40% of Japanese people have the inactive form of ALDH2, encoded as either heterozygous *ALDH2*1/2*2* or homozygous *ALDH2*2*.[7] Most homozygous carriers of this allele (*ALDH2*2/2*2*) are infrequent drinkers, because the enzyme deficiency would cause a strong facial flushing response, physical discomfort and severe toxic reactions. However, the heterozygous genotype (*ALDH2*1/2*2*) contributes substantially to the development of esophageal cancer[8,9] as a result of the higher levels of acetaldehyde in the blood and saliva after alcohol drinking.[10] In a recent study, we showed that *ALDH2*1/2*2* genotype increased the risk of pancreatic cancer among male smokers and that the drinking habit was not associated with pancreatic cancer.[11] However, the positive association of *ALDH2* polymorphism and pancreatic cancer in female subjects was not elucidated because of the small number of female subjects.

In contrast, it has been reported that obesity, insulin resistance, drinking alcohol, eating red meat and a high fat diet enhance the incidence of colon cancer.[12,13] Thus, in the present study, we examined the association between alcohol, pancreatic cancer (especially in female subjects), colon cancer and *ALDH2* gene polymorphism.

Methods

In the present study, patients with pancreatic cancer consisted of 112 male subjects (mean age, 62 years; age range, 41–80 years) and 75 female subjects (mean age, 66 years; age range, 43–93 years) who had been consecutively hospitalized (2001–2006) at the National Kyushu Cancer Center in Fukuoka, Japan. Pancreatic cancer was diagnosed clinically by imaging techniques including ultrasound, computed tomography (CT) scanning and magnetic resonance tomography, and was confirmed by histological examination.

For the colon cancer subjects, we investigated 513 Japanese patients with positive occult blood in their stools. The 221 male subjects and 292 female subjects had been consecutively hospitalized at the Tokyo Metropolitan Geriatric Hospital. Colonoscopy, gastroscopy and ultrasound examinations were carried out.

Subjects who consumed more than 5 g of ethanol per day were judged as having a drinking habit. The smoking status classifications were: current smoker, ex-smoker and never smoked. Only current smokers were judged as having a smoking habit.

The age-matched control subjects consisted of 1050 male participants (mean age, 59 years; age range, 40–79 years) and 1020 female participants (mean age 58 years; age range, 40–79 years) in the NILS Longitudinal Study of Aging (LSA).[14]

The genotype of the *ALDH2* gene was determined by a mismatched PCR-restriction fragment length polymorphism (RFLP) method reported previously.[15]

The present study was approved by the Ethics Committees of the National Kyushu Cancer Center, of the National Institute for Longevity Sciences (NILS), Tokyo Metropolitan Geriatric Hospital and of the Tokyo Metropolitan Institute of Gerontology. Written informed consent was obtained from each subject.

The statistical associations between pancreatic cancer and five covariates (sex, age, drinking, smoking and *ALDH2* polymorphism) were evaluated using the logistic regression model or a 2×2 χ^2-test. Independent prognostic factors were determined through the stepwise variable selection procedure. A value of $P < 0.05$ was considered to be statistically significant and all P-values are reported as two-tailed.

Results

Pancreatic cancer patients

The frequency of smoking was found to be higher in pancreatic cancer patients than in control subjects regardless of sex (69.6% for pancreatic cancer patients and 38.2% for control subjects among males; 20.0% for pancreatic cancer patients and 6.9% for control subjects among females). Importantly, after adjusting the effects of drinking and smoking habits by multivariate analysis, we observed no sex difference between pancreatic cancer and control subjects (male *vs* female odds ratio [OR] = 1.06; $P = 0.74$) (Table 2).

The frequency of the active form of *ALDH2* (*ALDH2*1/2*1*) was lower in pancreatic cancer patients than in control subjects (47.6% *vs* 51.1%), whereas the frequency of the inactive heterotype (*ALDH2*1/2*2*) was higher in pancreatic cancer patients (43.3% *vs* 39.9%). The frequency of *ALDH2*2/2*2* was consistent (9.1% *vs* 9.0%). Overall, the distributions of *ALDH2* gene genotypes showed a statistically significant difference between the two groups ($P = 0.0043$) (Table 3).

We carried out stepwise logistic regression to determine independently prognostic factors, identifying three such factors: smoking status (smoker *vs* non-smoker, OR = 1.91; $P < 0.001$), age (65≤ *vs* >65, OR = 2.20; $P < 0.001$) and *ALDH2* (inactive *ALDH2*1/2*2 vs* active, OR = 1.62; $P = 0.003$) (Table 3). Neither drinking habit nor sex was a significant risk of pancreatic cancer. Thus,

Table 2 Smoking and/or drinking habits between pancreatic cancer patients and control subjects

Habit	Both smoking and drinking *n* (%)	Smoking alone *n* (%)	Drinking alone *n* (%)	Neither habit *n* (%)	Total
Male					
PCa	54 (48.2)*	24 (21.4)†	15 (13.4)	19 (17.0)	112
Control	294 (28.0)	107 (10.2)	419 (39.9)	230 (21.9)	1050
Female					
PCa	2 (2.7)	13 (17.3)	8 (10.7)	52 (69.3)	75
Control	25 (2.5)	46 (4.5)	227 (22.3)	722 (70.8)	1020

*The high percentage of male pancreatic cancer patients with both smoking and drinking habits. †A large percentage of pancreatic cancer patients were smokers among both male and female subjects. PCa, pancreatic cancer.

Table 3 Distribution of *ALDH2* gene genotypes in patients with pancreatic cancer and control subjects

Genotype	PCa ($n = 187$)	Control ($n = 2070$)
*ALDH2*1/2*1* (active)	89 (47.6%)	<1057 (51.1%)
*ALDH2*1/2*2* (inactive)	81 (43.3%)	>827 (39.9%)
*ALDH2*2/2*2* (inactive)	17 (9.1%)	186 (9.0%)

The difference between the wild-type genotype and the mutations (the sum of the inactive form) was tested by 2×2 χ^2-test. *The frequency was significantly lower compared with that in control subjects ($\chi^2 = 5.65$, df = 1, $P = 0.018$). Reproduced from reference 11. PCa, pancreatic cancer.

Table 4 Results of multivariate analysis

Categories in comparison	Estimated odds ratio	*P*-value
Smoker *vs* non-smoker	1.91	<0.001
Age >65 years *vs* <65 years	2.20	<0.001
ALDH2 inactive *vs* active	1.62	0.003

our analysis suggests that an *ALDH2* polymorphism (*ALDH2*1/2*2*) is associated with pancreatic cancer with a 1.6-fold increase of morbidity risk. We are further able to estimate the relative risk (odds ratio) of pancreatic cancer in the patient subgroups defined by these three factors. Specifically, the odds ratio of smoking patients over 65 years of age with an *ALDH2* polymorphism (*ALDH2*1/2*2*) is estimated to be more than 7-fold higher than that of non-smoking patients under 65 years of age with the active form of ALDH2 (Table 4).

Colon cancer patients

The mean age was high (over 75 years), because the Tokyo Metropolitan Geriatric Hospital had been established for older patients. A high number of female subjects, especially more than 80 years of age, compared with male subjects might reflect the longer lifespan of females in Japan.

The frequency of male subjects who had both smoking and drinking habits was merely 19.9% (44/221); it was significantly lower compared with middle-aged subjects (28.0%).[11,14] The frequency of male subjects who had a smoking habit alone was 20.4% (45/221) and it was higher compared with middle-aged subjects (10%). Drinking habit alone was 22.6% (50/221); it was lower than the middle-aged subjects (39.2%). In female subjects, these frequencies were not different from the middle-aged subjects (2.5% for both smoking and drinking, 4.5% for smoking alone and 22.3% for drinking alone).

Benign colon polyps were observed in 211 patients (110 males and 101 females), endoscopically defined colon cancer was observed in 20 subjects (13 males and 7 females), cancer in adenoma was in 29 subjects (18 males and 11 females), the rest without colon polyps or cancer were 252 (84 males and 138 females) (Table 5). The remaining subjects without colon polyps or cancer might not be healthy because some of them has illnesses such as gastric ulcers or gallstones.

The frequency of smoking habits tended to be higher and drinking habits tended to be lower in patients with colon cancer compared with the patients without cancer. However, age, body mass index (BMI), the frequency of smoking and/or drinking habits, or the distribution of *ALDH2* genotypes did not differ significantly among these four groups (Table 6).

Table 5 Clinical features of colon cancer, colon polyps and others

	Colon cancer ($n = 49$)	Colon polyp ($n = 211$)	Others ($n = 252$)
Age (years)	76.9 ± 1.2	77.3 ± 0.4	77.6 ± 0.4
Body mass index	22.4 ± 0.5	22.6 ± 0.2	22.5 ± 0.2
Alcohol	12 (24%)	65 (31%)	80 (32%)
Smoking	19 (39%)	49 (23%)	43 (17%)
Alcohol and smoking	5 (10%)	23 (11%)	22 (9%)

Values are the mean ± SE.

Table 6 Distributions of *ALDH2* genotypes in patients with colon cancer, colon polyps and others

	Colon cancer ($n = 49$)	Colon polyp ($n = 211$)	Others ($n = 252$)
*ALDH2*1/2*1*	24 (49.0%)	120 (56.9%)	112 (44.4%)
*ALDH2*1/2*2*	22 (44.9%)	81 (38.4%)	125 (49.6%)
*ALDH2*2/2*2*	2 (4.0%)	10 (4.7%)	15 (6.0%)

Discussion

In Japan, pancreatic cancer ranks as the fifth most common cause of cancer deaths, and the five-year survival rate of its victims is less than 10%.[16] Smoking is a well-documented risk factor for the development of pancreatic adenocarcinoma.[1] In contrast, alcohol intake has not been firmly established as being causally related or unrelated to pancreatic cancer.[17,18] Heavy alcohol intake might cause chronic pancreatitis. Alcoholic pancreatitis, which accounts for 55.5% of pancreatitis cases, is the most common type in Japanese men (68.5%).[19] Chronic pancreatitis has been indicated as a risk factor for pancreatic cancer.[20–23]

Orally ingested ethanol is metabolized by alcohol dehydrogenase (ADH), and the first metabolite is acetaldehyde. Most of the acetaldehyde generated during alcohol metabolism is eliminated by liver mitochondrial ALDH2, which converts the acetaldehyde into acetic acid. ALDH2 is responsible for metabolizing the acetaldehyde produced from ethanol into acetate.

The Japanese population is deficient in ALDH2 because of the high frequency of a mutant allele in the *ALDH2* gene (*ALDH2*2*). The *ALDH2*2* allele encodes a Glu-to-Lys amino acid substitution at the 14th and last codon (GAA, GAG to AAA, AAG). More than 40% of Japanese people have the inactive form of ALDH2, encoded either as heterozygous *ALDH2*1/2*2*. or as homozygous *ALDH2*2*.[7] Most homozygous carriers of this allele (*ALDH2*2/2*2*) are infrequent drinkers because the enzyme deficiency causes a strong facial flushing response, physical discomfort and severe toxic reactions. However, the heterozygous genotype (*ALDH2*1/2*2*) contributes substantially to the development of esophageal cancer, possibly as a result of the higher levels of acetaldehyde in the blood and saliva after drinking alcohol.[8–10,24]

The inactive form of ALDH2 is known to produce high levels of acetaldehyde, an animal carcinogen, which accumulates in the blood and[ism.25] In a previous study by Harada *et al.*, acetaldehyde concentrations in the blood were significantly higher in subjects with inactive ALDH2 than in those with active ALDH2 after 0.5 g/kg ethanol was given orally (35.3 ± 12.8 μmol/L in 19 subjects with inactive ALDH2 *vs* 2.1 ± 1.7 μmol/L in 25 subjects with active ALDH2), whereas ethanol concentrations were comparable (10 mmol/L).[26]

We previously reported that smoking enhances the risk of pancreatic cancer in Japanese male subjects with *ALDH2*1/2*2*.[11] However, we were unable to identify a similar significant association in female subjects because of the small number of patients. In the present report, we concluded that *ALDH2*1/2*2* is an independent risk factor of pancreatic cancer and that there is no sex difference

Risk factors for colorectal cancer include family history of colorectal cancer or adenoma, previous history of colorectal cancer, colorectal adenoma, inflammatory bowel disease, smoking, high-fat food consumption, excess total energy intake, physical inactivity and obesity.[27–32] Metabolic syndrome is a complex metabolic disease characterized by the constellation of glucose intolerance, obesity, hypertension and dyslipidemia. The core elements shared by obesity, diabetes mellitus and metabolic syndrome are hyper insulinemia and insulin resistance, which play a major role in the

carcinogenic process in breast, prostate, endometrial and colorectal cancers.[33–35] The association between alcohol consumption and colorectal cancer has been reported. Regular consumption of approximately 50 g of alcohol per day increased the relative risk of about 1–4 for colorectal cancer compared with that in non-drinkers.[36,37]

WHO/FAO2003 reported that obesity and alcohol were positive risks for colon cancer.[38,39] In the present study, we could not find a significant association between obesity, alcohol and colon cancer. Two reasons were proposed to explain the difference between the WHO report and our observation. First, the numbers of subjects might be too small in the present study. Second, the distribution of age might be different from the other studies; Tokyo Metropolitan Geriatric Hospital was originally established for aged patients, that is, more than 65 years of age, and the mean age of patients was approximately 82 years. Although the frequency of drinking and/or smoking was lower and/or higher compared with the middle-aged group, we could not confirm their drinking and smoking habits in the past.

In conclusion, inactive ALDH2 might be a positive risk for pancreatic cancer, but not for colon cancer.

Conflicts of interest

None.

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Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan

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BACKGROUND: A British randomised study of gemcitabine plus cisplatin (GC) combination showed promising results in biliary tract cancer (BTC) patients. In our study, we evaluated the efficacy and safety of this combination compared with gemcitabine alone (G) in Japanese BTC patients.

METHODS: Overall, 84 advanced BTC patients were randomised to either cisplatin 25 mg m^{-2} plus gemcitabine 1000 mg m^{-2} on days 1, 8 of a 21-day cycle (GC-arm), or single-agent gemcitabine 1000 mg m^{-2} on days 1, 8 and 15 of a 28-day cycle (G-arm). Treatments were repeated for at least 12 weeks until disease progression or unacceptable toxicity occurred, up to a maximum of 48 weeks.

RESULTS: A total of 83 patients were included in the analysis. For the GC and G-arms, respectively, the 1-year survival rate was 39.0 *vs* 31.0%, median survival time 11.2 *vs* 7.7 months, median progression-free survival time 5.8 *vs* 3.7 months and overall response rate 19.5 *vs* 11.9%. The most common grade 3 or 4 toxicities (GC-arm/G-arm) were neutropenia (56.1%/38.1%), thrombocytopenia (39.0%/7.1%), leukopenia (29.3%/19.0%), haemoglobin decrease (36.6%/16.7%) and γ-GTP increase (29.3%/35.7%).

CONCLUSIONS: Gemcitabine plus cisplatin combination therapy was found to be effective and well tolerated, suggesting that it could also be a standard regimen for Japanese patients.

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Keywords: combination chemotherapy; gemcitabine; cisplatin; biliary tract cancer

Although biliary tract cancer (BTC) is a rare type of cancer throughout the world, it is more prevalent in East Asia and Latin America than in other countries (Matsuda and Marugame, 2007; Randi *et al*, 2009). According to 'Demographic Statistics in Japan (2009)' (compiled by the Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour, and Welfare (MHLW)), the number of deaths due to BTC was 17311 in 2007, making this cancer the sixth leading cause of cancer death in Japan.

Despite great progress in diagnostic imaging, most cases of BTC are diagnosed as advanced and inoperable. Even if the tumour is not locally advanced, the primary tumour site is often contiguous with vital organs such as the liver, pancreas, or duodenum, or with major vessels such as the portal vein or hepatic artery. This anatomical peculiarity precludes resection of tumours in many cases. Furthermore, even if curative-intent surgical resection is performed, the cancer often relapses due to its invasive nature and its anatomical characteristics.

Systemic chemotherapy is usually indicated for patients with unresectable, advanced BTC or for those who have relapsed after operation; however, no standard treatment has yet been established for such patients. Gemcitabine hydrochloride is a deoxycytidine derivative that inhibits DNA elongation through intracellular phosphorylation of ribonucleotide reductase. In Japan, a single-arm Phase II study in patients with unresectable BTC confirmed that gemcitabine monotherapy had moderate efficacy and manageable toxicity, both of which were comparable with approved treatments for other cancers (Okusaka *et al*, 2006).

As gemcitabine had also been found to exhibit synergistic effects on cytotoxic activity *in vitro* and *in vivo* when combined with cisplatin (Peters *et al*, 1995; Bergman *et al*, 1996), clinical studies were conducted in various cancers with this combination. Results from these studies eventually led to use of the gemcitabine plus cisplatin (GC) combination as one of the standard treatments for non-small cell lung cancer and bladder cancer.

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The combination of GC has also been studied by many researchers for the treatment of BTC (Park *et al*, 2006; Eckel and Schmid, 2007; Pasetto *et al*, 2007; Lee *et al*, 2008). So far, the largest randomised Phase III study has been the recent UK ABC-02 study, in which the efficacy and safety of gemcitabine 1000 mg m^{-2} alone *vs* the combination of gemcitabine 1000 mg m^{-2} plus cisplatin 25 mg m^{-2} was evaluated by British research groups (Cancer Research UK and University College London). That study was initiated as a randomised phase II study with gemcitabine alone *vs* GC (UK ABC-01 study) and then was expanded to a phase III study (ABC-02 study) (Valle *et al*, 2009a, b).

Our study was planned to follow-up on an earlier study of gemcitabine monotherapy conducted in Japanese BTC patients (Okusaka *et al*, 2006). Given the encouraging results from the UK ABC-01 study, we conducted this study to (1) evaluate both gemcitabine monotherapy and the GC combination in Japanese BTC patients, and (2) determine whether benefits similar to those observed in the UK study could be obtained for the combination regimen.

The primary objective of the study was to compare the 1-year survival rate in patients with BTC who received one of these two therapies. The secondary objectives included response rate, progression-free survival (PFS) and assessment of safety.

MATERIALS AND METHODS

Study design

This was a multicentre, randomised phase II study to evaluate the efficacy and safety of GC combination compared with single-agent gemcitabine in chemotherapy-naive patients with locally advanced or metastatic BTC. Patients were randomised to either single-agent gemcitabine 1000 mg m^{-2} on days 1, 8 and 15 of a 28-day cycle (G-arm) or cisplatin 25 mg m^{-2} followed by gemcitabine 1000 mg m^{-2} on days 1, 8 of a 21-day cycle (GC-arm). Randomisation was stratified by primary site (gallbladder cancer or other BTC) and the presence or absence of primary tumour.

Eligibility criteria

Eligible patients met the following criteria: histologically confirmed unresectable locally advanced or metastatic cancer of the biliary tract; no history of earlier chemotherapy; performance status of 0 or 1; a life expectancy of at least 3 months; at least 20 years of age at the time of study entry; adequate function of major organs (haemoglobin $\geqslant$ 10 g per 100 ml, white blood cells $\geqslant$ 3000/mm^3, neutrophils $\geqslant$ 1500/mm^3, platelets $\geqslant$ 100 000/mm3, AST/ALT/ALP $\leqslant$ 3 times upper limit of normal (ULN), total bilirubin $\leqslant$ 2 times ULN, $\leqslant$ 3 times ULN for patients with obstructive jaundice or metastases to the liver, serum creatinine $\leqslant$ 1.5 times ULN, creatinine clearance or 24-h creatinine clearance $\geqslant$ 45 ml min^{-1}).

This study followed the ethical principles that have their origins in the Declaration of Helsinki, and was conducted in accordance with the protocol, the 'ordinance on Good Clinical Practice' and related regulations. Written informed consent was obtained from all patients who were considered eligible for participation in this study before enrolment. The Efficacy and Safety Evaluation Committee, an independent review board, was consulted if any efficacy and safety issues arose in the study.

Study treatment

The assigned treatment was given for a minimum of 12 weeks (at least four cycles in the GC-arm and three cycles in the G-arm) and continued to a maximum of 48 weeks (up to 16 cycles in the GC-arm and up to 12 cycles in the G-arm), unless disease progression (PD) was evident, an intolerable adverse event occurred or the patient was required to withdraw from the study.

Efficacy and safety assessment

All patients who received at least 1 dose of the study drug were included in the efficacy and safety assessment. Response rate was evaluated according to the Response Evaluation Criteria in Solid Tumors. Evaluation of tumours after patient randomisation was performed every 6 weeks until PD. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE v3.0).

Statistical design and analysis

The sample size was calculated by the selection method of Simon (Simon *et al*, 1985), which is based on the proposition that GC combination therapy is selected if the 1-year survival rate for the GC-arm is higher than that for the gemcitabine arm. We assumed a 1-year survival rate of 25% for the G-arm and 35% for GC-arm (Okusaka *et al*, 2006; Park *et al*, 2006). With these assumptions, 30 patients per arm were needed to appropriately select the combination therapy with a probability of $\geqslant$ 80%. To optimise safety and efficacy information, the sample size was set to 42 patients per arm.

The Kaplan–Meier method was used to estimate 1-year survival (primary outcome), PFS and 6-month PFS rates (secondary outcomes) for each treatment arm; 95% confidence intervals (CIs) were calculated. A Cox proportional hazards model was used to calculate the hazard ratio, 95% CI and its two-tailed *P*-value. Fisher's exact test was used to compare the patient characteristics, response and disease control rates, and toxicities between the two treatment arms. The exact CIs were calculated based on binomial distributions.

RESULTS

Patients

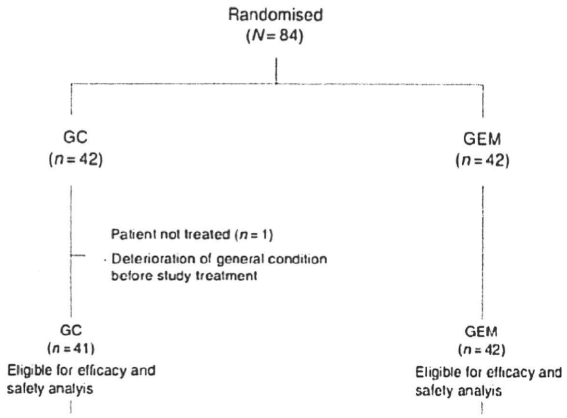
This study was carried out from September 2006 to October 2008 at nine study centres in Japan. Eighty-four patients were randomised to either gemcitabine monotherapy (G-arm) or GC combination (GC-arm). One patient assigned to the GC-arm was not treated because the general condition of the patient deteriorated before study treatment. All of the remaining 83 patients, 41 in the GC-arm and 42 in the G-arm, received at least 1 dose of study treatment. Efficacy and safety were evaluated for each of these 83 patients (Figure 1). Demographic variables (Table 1) were well balanced between the two treatment arms, except for patients with ampullary carcinoma (4 in GC-arm, 0 in G-arm).

Drug exposure and duration of the treatments

A total of 247 (median 6.0) and 203 (median 4.0) cycles were administered in the GC-arm and G-arm, respectively. Relative dose intensities were 78.9% for gemcitabine and 79.0% for cisplatin in the GC-arm, and 87.4% for gemcitabine in the G-arm. Three patients in the GC-arm and two patients in the G-arm completed 48 weeks treatment.

Efficacy

A total of 83 patients were evaluable for tumour response according to the protocol, 41 in the GC-arm and 42 in the G-arm. No complete tumour responses were observed. In total, eight patients in the GC-arm had a partial response (PR) compared with five patients in the G-arm (PR 19.5 *vs* 11.9%). In addition,



Reasons for discontinuation	GC arm	GEM arm
Progression of disease	25	34
Unable to start next cycle	4	0
Adverse event	7	3
Patient decision	1	3
Physician decision	1	0
Completed study (48 weeks)	3	2

Figure 1 CONSORT diagram. Disposition of patients. GC = gemcitabine–cisplatin combination; GEM = gemcitabine alone.

20 patients had stable disease in the GC-arm *vs* 16 patients in the G-arm (SD 48.8 *vs* 38.1%). The disease control rate (CR + PR + SD) was 68.3% (95% CI: 51.9, 81.9) *vs* 50.0% (95% CI: 34.2, 65.8) in favour of the combination therapy. The 1-year survival rate (39.0 *vs* 31.0%), median survival time (11.2 months *vs* 7.7 months) and median PFS (5.8 months *vs* 3.7 months) were better for the GC-arm *vs* G-arm (Figure 2). The hazard ratio between the GC and G-arms was 0.69 (95% CI: 0.42, 1.13) for overall survival (OS) and 0.66 (95% CI: 0.41, 1.05) for PFS (Table 2).

As shown in Table 3, the prognosis for patients with gallbladder cancer was worse than that for patients with non-gallbladder cancer; however, the median survival times were longer with the GC combination in gallbladder cancer patients (9.1 months *vs* 6.7 months), as well as in patients with non-gallbladder cancer (13.0 months *vs* 8.0 months). The prognosis for patients with primary tumours was worse than that for patients without primary tumours; however, the GC therapy showed longer median survival time in both patient subgroups (9.4 months *vs* 7.4 months in the patients with primary tumours, 16.1 months *vs* 12.7 months in the patients without primary tumours).

Safety

All adverse events observed in this study were predictable and manageable based on the safety profile of GC. As shown in Table 4, the most common grade 3 or higher adverse events (≥25%) were neutropenia (56.1%), thrombocytopenia (39.0%), haemoglobin decrease (36.6%), RBC decrease (34.1%), leukopenia (29.3%) and γ-GTP increase (29.3%) in the GC-arm, and neutropenia (38.1%) and γ-GTP increase (35.7%) in the G-arm. The incidence of haematotoxicity was higher in the GC-arm; grade 3 or more serious C-reactive protein increase was detected only in the monotherapy arm.

Table 1 Patient characteristics

Characteristic	GC (*N* = 41) *n* (%)	GEM (*N* = 42) *n* (%)	*P*-value
Gender			
Male	18 (43.9)	21 (50.0)	0.662
Female	23 (56.1)	21 (50.0)	
Age (year)			
Median	65.0	66.5	0.0812[a]
Range	43–80	49–78	
PS			
0	34 (82.9)	28 (66.7)	0.129
1	7 (17.1)	14 (33.3)	
Primary tumour sites			
Extrahepatic bile duct	8 (19.5)	11 (26.2)	0.239
Intrahepatic bile duct	14 (34.1)	14 (33.3)	
Gallbladder	15 (36.6)	17 (40.5)	
Ampulla	4 (9.8)	0 (0.0)	
Metastatic sites			
Liver	22 (53.7)	20 (47.6)	0.663
Regional lymph nodes	23 (56.1)	28 (66.7)	0.372
Distant lymph nodes	19 (46.3)	18 (42.9)	0.827
Lung	8 (19.5)	7 (16.7)	0.782
Peritoneum	7 (17.1)	7 (16.7)	1.000
Bone	0 (0.0)	1 (2.4)	1.000
Others	3 (7.3)	3 (7.1)	1.000
Initial onset or recurrence			
Initial onset	30 (73.2)	32 (76.2)	0.804
Recurrence after surgery	11 (26.8)	10 (23.8)	
Histological type			
Adenocarcinoma	39 (95.1)	41 (97.6)	0.616
Adenosquamous cancer	2 (4.9)	1 (2.4)	
Disease stage (gallbladder cancer, extrahepatic bile duct cancer, ampulla cancer)			
IIA	0 (0.0)	0 (0.0)	1.000
IIB	3 (7.3)[b]	2 (4.8)[b]	
III	2 (4.9)	2 (4.8)	
IV	16 (39.0)	17 (40.5)	
Recurrence after surgery	6 (14.6)	7 (16.7)	
Disease stage (intrahepatic bile duct cancer)			
II	0 (0.0)	1 (2.4)[c]	0.389
IIIA	0 (0.0)	1 (2.4)	
IIIB	0 (0.0)	0 (0.0)	
IIIC	0 (0.0)	2 (4.8)	
IV	9 (22.0)	7 (16.7)	
Recurrence after surgery	5 (12.2)	3 (7.1)	
Biliary drainage			
No	25 (61.0)	24 (57.1)	0.824
Yes	16 (39.0)	18 (42.9)	
Previous therapy			
No	30 (73.2)	28 (66.7)	0.855
Surgery	11 (26.8)	12 (28.6)	
Radiotherapy	0 (0.0)	1 (2.4)	
Surgery and radiotherapy	0 (0.0)	1 (2.4)	

Abbreviations: GC = gemcitabine and cisplatin; GEM = gemcitabine; PS = performance status. [a]*t*-test. [b]Patients were diagnosed as having unresectable disease with marked regional node metastases involving the proper hepatic artery and/or main portal vein.

There were no treatment related deaths. Most of the patients recovered from the above adverse events by reducing or discontinuing the study treatment.

Post-study chemotherapy

Thirty patients in the GC-arm received post-study chemotherapy including S-1, tegaful/gimeracil/oteracil potassium (19 patients), gemcitabine (10 patients) and tegaful/uracil (1 patient). In the G-arm, 33 patients received post-study chemotherapy including S-1 (20 patients), gemcitabine (11 patients), cisplatin/fluorouracil (1 patient) and doxorubicin/tegaful/uracil (1 patient).

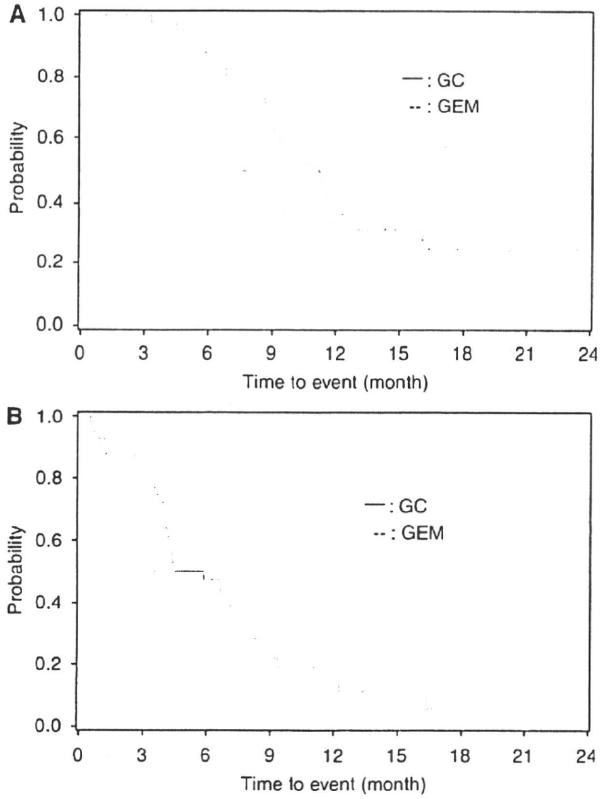


Figure 2 Kaplan–Meier curve of overall survival and progression-free survival. (**A**) Overall survival. (**B**) Progression-free survival. GC = gemcitabine–cisplatin combination; GEM = gemcitabine alone; CI = confidence interval.

DISCUSSION

Although this study (BT22 study) showed that gemcitabine monotherapy and the GC combination were both active in Japanese patients with advanced BTC, a superior benefit was obtained with the combination treatment. In the GC/G-arms, the 1-year survival rate was 39.0%/31.0%, median survival time was 11.2/7.7 months and median PFS time was 5.8/3.7 months (Table 2).

The UK ABC-02 study, which was conducted with the same dose and regimen as this study (Valle *et al*, 2009b), showed a similar benefit for the GC combination. The respective median survival/PFS times in that study were 11.7/8.5 months in their GC-arm, and 8.2/6.5 months in their G-arm.

The hazard ratios reported in the ABC-02 study for OS (0.68, 95% CI: 0.53, 0.86) and PFS (0.70, 95% CI: 0.56, 0.88) compared well with the respective values from our study: 0.69 (95% CI: 0.42, 1.13) and 0.66 (95% CI: 0.41, 1.05). As the number of patients was based on Simon's selection method (Simon *et al*, 1985), this study was not designed to compare and identify statistical significant differences between the two treatment arms. These hazard ratios

Table 3 Overall survival time by stratification factor

Median survival time (months) (95% CI)	GC ($N = 41$)	GEM ($N = 42$)	*P*-value
Tumour site			
Gallbladder	9.1 (6.9, 11.6)	6.7 (4.2, 11.0)	0.675
Non-gallbladder	13.0 (9.2, ***)	8.0 (6.1, 16.0)	0.110
Primary tumour			
Presence of primary tumour	9.4 (8.7, 11.6)	7.4 (5.9, 8.5)	0.253
Absence of primary tumour	16.1 (12.3, ***)	12.7 (6.5, ***)	0.389

Abbreviations: GC = gemcitabine and cisplatin; GEM = gemcitabine; CI = confidence interval. ***denotes upper limits are not available.

Table 2 Summary of time-to-event end points: overall response and survival

	GC ($N = 41$) *n* (%)	GEM ($N = 42$) *n* (%)	*P*-value
Overall response rate			
Complete response (CR)	0 (0.0)	0 (0.0)	
Partial response (PR)	8 (19.5)	5 (11.9)	
Stable disease (SD)	20 (48.8)	16 (38.1)	
Progressive disease (PD)	9 (22.0)	17 (40.5)	
Not evaluable (NE)	4 (9.8)	4 (9.5)	
Response rate (95% CI)	19.5% (8.8, 34.9)	11.9% (4.0, 25.6)	0.380
Disease control rate (CR+PR+SD) (95% CI)	68.3% (51.9, 81.9)	50.0% (34.2, 65.8)	0.119
Overall survival			
1-year survival rate (95% CI)	39.0% (23.7, 54.4)	31.0% (17.0, 44.9)	
Median survival time (95% CI)	11.2 months (9.1, 12.5)	7.7 months (6.1, 11.0)	
Hazard ratio (95% CI)	0.69 (95% CI: 0.42, 1.13)		0.139
Progression-free survival (PFS)			
Median PFS (95% CI)	5.8 months (4.1, 8.2)	3.7 months (2.1, 5.3)	
Hazard ratio (95% CI)	0.66 (95%CI: 0.41, 1.05)		0.077
6-Months PFS rate (95% CI)	47.4% (31.4, 63.4)	27.7% (14.0, 41.5)	

Abbreviations: GC = gemcitabine and cisplatin; GEM = gemcitabine; CI = confidence interval.

Table 4 Summary of maximum toxicity grades[a] (incidence ⩾30%)

	GC ($N = 41$)			GEM ($N = 42$)			
	Maximum toxicity grade			Maximum toxicity grade			
Events	**Grade 3 (%)**	**Grade 4 (%)**	**All grades (%)**	**Grade 3 (%)**	**Grade 4 (%)**	**All grades (%)**	***P*-value**
Haematological							
WBC count decreased	29.3	0	87.8	19.0	0	69.0	0.061
Haemoglobin decreased	26.8	9.8	85.4	9.5	7.1	85.7	1.000
Neutrophil count decreased	39.0	17.1	82.9	28.6	9.5	69.0	0.200
Platelet count decreased	26.8	12.2	80.5	4.8	2.4	76.2	0.791
RBC decreased	34.1	0	75.6	14.3	0	78.6	0.798
Haematocrit decreased	4.9	0	58.5	0	0	54.8	0.826
Non-haematological							
Anorexia	0	0	80.5	4.8	0	61.9	0.090
Nausea	0	0	68.3	0	0	42.9	0.027
Fatigue	0	0	58.5	2.4	0	50.0	0.511
AST increased	17.1	0	53.7	14.3	2.4	52.4	1.000
ALT increased	24.4	0	51.2	16.7	0	52.4	1.000
Vomiting	0	0	48.8	0	0	23.8	0.023
GGT increased	29.3	0	46.3	31.0	4.8	50.0	0.827
Pyrexia	0	0	43.9	4.8	0	57.1	0.190
LDH increased	0	0	36.6	0	0	35.7	1.000
Constipation	0	0	36.6	0	0	33.3	0.820
ALP increased	7.3	0	31.7	16.7	0	40.5	0.495
Weight decreased	0	0	31.7	0	0	31.0	1.000
Diarrhoea	2.4	0	31.7	0	0	26.2	0.634
Blood sodium decreased	17.1	0	31.7	9.5	0	19.0	0.214
C-reactive protein increased	0	0	26.8	7.1	0	52.4	0.025

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GC = gemcitabine and cisplatin; GEM = gemcitabine; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase; RBC = red blood cell; WBC = white blood cell. [a]Events were graded according to CTCAE v3.0.

strongly suggest that the GC combination has superior benefit compared with single-agent gemcitabine, even though there were no statistical significant differences in survival and PFS between the two arms in our study.

Although there have been many single-arm Phase II studies of the GC combination for BTC (Thongprasert *et al*, 2005; Kim *et al*, 2006; Charoentum *et al*, 2007; Meyerhardt *et al*, 2008; Valle *et al*, 2009a), these results have never been distilled to one fixed dose and regimen of GC. Many previous studies of GC combination reported relatively higher response rates, but with more serious treatment-related adverse events (Thongprasert *et al*, 2005; Kim *et al*, 2006; Charoentum *et al*, 2007; Meyerhardt *et al*, 2008). In the phase II study conducted by Thongprasert *et al* (2005), 17.85% of the patients who were treated with the GC combination required dose reduction, and in another Phase II study recently conducted by Meyerhardt *et al* (2008), dose reductions and study withdrawals were required for 50% of the patients who received the combination therapy. In our study, we also observed more frequent adverse events with the doublet (Table 4). However, as shown in Figure 1, only seven patients (17%) discontinued from the study because of adverse events and four patients (9.7%) required dose adjustments in the GC-arm.

Overall, the toxicity observed in this study was manageable. Although interstitial pneumonia was detected in one patient from each of the arms, both patients recovered with appropriate treatment. One grade 3 renal failure and one grade 2 peripheral neuropathy were observed in GC-arm, in line with similar events seen in previous studies of the GC combination (Thongprasert *et al*, 2005; Kim *et al*, 2006; Charoentum *et al*, 2007; Meyerhardt *et al*, 2008; Valle *et al*, 2009a). It is to be noted that despite the higher incidence of haematotoxicity in patients receiving the combination therapy, drug-caused myelosuppression did not result in febrile neutropenia or bleeding. Grade 3 or greater increases in C-reactive protein were observed only in the gemcitabine monotherapy-arm, also suggesting that the combination therapy did not increase neutropenic infections.

In this study, we stratified patients into those with gallbladder cancer and those with other BTCs. Gallbladder cancer has been reported to have a different biological behaviour (Kim *et al*, 2006; Doval *et al*, 2004; Jarnagin *et al*, 2006); furthermore, a pooled analysis by Eckel and Schmid (2007) revealed a higher response rate to chemotherapy and shorter OS for gallbladder cancer compared with other BTCs. As shown in Table 3, patients with gallbladder cancer showed worse survival than patients with other BTCs, this being consistent with previous reports (Eckel and Schmid, 2007; Wagner *et al*, 2009). It is important to note that median survival times were longer with the GC combination in patients with gallbladder cancer (9.1 months *vs* 6.7 months), as well as in patients with non-gallbladder cancer (13.0 months *vs* 8.0 months), suggesting that the combination therapy has greater benefit than monotherapy in gallbladder cancer and other BTC patients.

Another stratification factor used for this study was the presence or absence of a primary tumour, not a commonly used stratification factor in clinical trials for advanced BTC. Locally advanced or metastatic cancer, the stratification factor used in the UK ABC-01 and UK ABC-02 studies, is more commonly used, as both of these have been shown to affect OS in advanced BTC (Park *et al*, 2009). However, considering the importance of surgical resection of the primary tumour, we decided to use this as a stratification factor for patients in this study. As shown in Table 3, patients with primary tumours showed remarkably worse survival than patients without primary tumours. However, because of the limited number of patients in our subanalyses, the results should be viewed with caution, and the usefulness of this prognostic factor should be evaluated in future studies. We will continue our efforts

in collaboration with the UK ABC-02 study group to identify prognostic factors in a larger population, which may significantly affect clinical studies in BTC.

Despite the heterogeneous nature of BTC and the ethnic differences reported for this tumour type (Goodman and Yamamoto, 2007; Aljiffry *et al*, 2009), the outcomes from this study showed striking similarity with the large-scale phase III study (UK ABC-02) results. This suggests that cisplatin 25 mg m^{-2} plus gemcitabine 1000 mg m^{-2} on days 1 and 8 of a 21-day cycle would be beneficial in the treatment of advanced BTC.

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Conflict of interest

TO, KN, NM, SO, SK and JF have received honoraria, and YN, MK, JF and SN are employed by Eli Lilly Japan.

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Phase II study of erlotinib plus gemcitabine in Japanese patients with unresectable pancreatic cancer

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Erlotinib combined with gemcitabine has not been evaluated in Japanese patients with unresectable pancreatic cancer. This two-step phase II study assessed the safety and pharmacokinetics of erlotinib 100 mg/day (oral) plus gemcitabine 1000 mg/m^2 (i.v. days 1, 8, 15) in a 28-day cycle in the first step, and efficacy and safety in the second step. The primary end-point was safety. One hundred and seven patients were enrolled (first step, n = 6; second step, n = 101). The most common adverse event was RASH (compiled using the preferred terms rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash) in 93.4% of patients. One treatment-related death occurred. While interstitial lung disease-like events were reported in nine patients (8.5%; grade 1/2/3, 3.8/2.8/1.9%), all patients recovered or improved. The median overall survival, the 1-year survival rate and median progression-free survival were 9.23 months, 33.0% and 3.48 months, respectively. The overall response and disease control rates were 20.3% and 50.0%, respectively. In Japanese patients with unresectable pancreatic cancer, erlotinib plus gemcitabine had acceptable toxicity and efficacy that was not inferior to that seen in Western patients. (*Cancer Sci*, doi: 10.1111/j.1349-7006.2010.01810.x, 2010)

Approximately 232 000 individuals are diagnosed with pancreatic cancer worldwide each year, with an annual death rate estimated at 227 000.[1] In Japan, approximately 22 000 new cases were reported in 2005.[2] Furthermore, data from 2007 show that around 24 000 individuals in Japan died from pancreatic cancer, making this tumor type the fifth leading cause of cancer-related death.[3] The majority of pancreatic cancer cases are diagnosed at an unresectable stage when prognosis is extremely poor.

Current treatment for advanced pancreatic cancer is based on systemic chemotherapy with gemcitabine. Single-agent gemcitabine has been shown to extend median overall survival (OS) to 5.65 months in chemonaïve patients compared with 4.41 months in patients who received fluorouracil.[4] Addition of other cytotoxic agents to gemcitabine has not demonstrated survival benefits over gemcitabine alone.[5–13] The potential of combining gemcitabine with biological agents in patients with advanced pancreatic cancer has also been evaluated in several phase III studies, but these trials failed to show a survival benefit.[14–19]

Epidermal growth factor receptor (EGFR)-mediated signaling is associated with various cellular processes, and the dysregulation of these processes is common in tumorigenesis.[20,21] Furthermore, EGFR is overexpressed in many tumors and its overexpression is often associated with poor prognosis.[22–26] EGFR tyrosine-kinase inhibitors (TKI, such as erlotinib) are used in the treatment of various types of solid tumors.

Erlotinib has demonstrated antitumor activity in pancreatic cell lines[27] and was subsequently assessed as a potential therapeutic agent in pancreatic cancer. In the PA.3 study (n = 569), the risk of death with erlotinib plus gemcitabine was reduced by 18% versus gemcitabine alone (hazard ratio [HR], 0.82; 95% confidence interval [CI], 0.69–0.99; P = 0.038 after adjustment for stratification factors), with a median OS of 6.24 months *vs* 5.91 months, respectively. Erlotinib plus gemcitabine combination therapy provided significant improvements in the 1-year survival rate (23% *vs* 17%; P = 0.023) and progression-free survival (PFS; HR 0.77; 95% CI, 0.64–0.92; P = 0.004).[28] As a result, this combination was approved for use in pancreatic cancer in many countries.

In Japanese patients with non-small-cell lung cancer (NSCLC), a phase II study has specifically shown that erlotinib monotherapy is well tolerated and has promising antitumor activity.[29] However, there are no data on the use of erlotinib combined with gemcitabine in Japanese patients with pancreatic cancer. This phase II study evaluated the safety and efficacy of erlotinib in combination with gemcitabine in Japanese patients with unresectable locally advanced or metastatic pancreatic cancer.

Methods

Patients. Patients aged 20–80 years with histological/cytological evidence of unresectable locally advanced or metastatic adenocarcinoma/adenosquamous carcinoma of the pancreas were eligible for inclusion in the present study. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, adequate hematological, renal and hepatic function and a life expectancy of at least 2 months. No more than one prior regimen for pancreatic cancer was permitted. Patients who had received prior gemcitabine and/or a TKI were excluded from participation, as were those who had previously been exposed to a human epidermal growth factor receptor 2 (HER2) or EGFR inhibitor. Other key

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Clinical trial registry: JAPIC Clinical Trials Information (see links below). http://rctportal.niph.go.jp/examDetail.php?center=3&center_seq=698 http://www.clinicaltrials.jp/user/cteDetail.jsp?clinicalTrialId=839&language=ja. Trial registration number: JapicCTI-060337.

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exclusion criteria were: symptomatic cerebral metastases; a concurrent lung disorder (such as idiopathic pulmonary fibrosis, interstitial lung disease [ILD] or pneumoconiosis); concurrent or previous drug-induced pneumonia; or a history of radiation to the chest.

The study complied with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all patients, and the protocol was approved by ethics committees at all participating institutions.

Study design and treatment. This was a phase II, multicentre, open-label, two-step study. In the first step, six patients were enrolled into the study and treated with oral erlotinib 100 mg/day on days 3–28, plus i.v. gemcitabine 1000 mg/m^2 on days 1, 8 and 15 in a 28-day cycle. The starting doses of erlotinib and gemcitabine were chosen in reference to the PA.3 study. Dose-limiting toxicities (DLT) were assessed in these study participants using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (NCI-CTCAE, National Cancer Institute, Bethesda, MD, USA). Dose-limiting toxicities were defined in conformity to the P1b study as follows:[30] (i) grade 4 decrease (i.e. to <500/mm^3) in neutrophil count >5 days; (ii) grade ≥3 decrease (i.e. to <1000/mm^3) in neutrophil count with associated fever (≥38.5°C); (iii) grade 4 decrease in platelet count (i.e. to <25 000/mm^3); (iv) any grade ILD; (v) grade 4 elevation of alanine transaminase (ALT)/aspartate transaminase (AST) levels, or grade 3 elevation of ALT/AST levels >7 days; (vi) grade ≥3 non-hematological toxicity (excluding rash, hyperglycemia, γ-GTP and events that were judged to be transient/had no effect on study continuation); and (vii) dose-reduction/interruption required due to persistent adverse events (AE), which meant that the second cycle could not be started.

If treatment-related DLT occurred in no more than two of the six patients, transition to the second step of the study was permissible with approval of the Data Safety and Monitoring Committee (DSMC). If DLT occurred in three or more patients, transition to the second step was limited to those cases that were judged to be safe for this study after the DSMC had evaluated the safety data of the patients with a DLT. In the second step, it was planned that 94 patients would be treated with the same dose as the first step. Treatment was continued until disease progression, death, unacceptable toxicity or patient/investigator request.

The primary end-point of the study was safety, with secondary end-points including OS, 1-year survival rate, PFS, overall response rate (ORR), disease control rate (DCR = complete response [CR] + partial response [PR] + stable disease), pharmacokinetics (PK) and correlation of *EGFR* mutation status with outcomes.

Toxicity evaluation. Adverse events were monitored and graded using NCI-CTCAE v3.0. Clinical and laboratory assessments were conducted throughout the study. Adverse events prespecified in the study to be monitored carefully were rash, diarrhea, vomiting, liver dysfunction and ILD-like events. Chest X-ray examination to assess pulmonary toxicity was conducted weekly until week 4 and every 2 weeks thereafter. In addition, chest computed tomography (CT) scan was performed every 4 weeks. The DSMC reviewed the images and clinical data associated with all potential ILD-like events. All ILD-like events were reported to be serious AE (SAE), regardless of the grade.

Efficacy evaluation. The tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) in patients who had at least one measurable target lesion. Tumors were measured using computed tomography (CT) at baseline and on day 22 of every two cycles thereafter. Median PFS, ORR and DCR were estimated by the extramural review. The relationship between efficacy and the severity of RASH (compiled using the preferred terms rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash) was also examined.

Pharmacokinetic evaluation. Pharmacokinetic evaluation of erlotinib and its O-desmethylated metabolite (OSI-420) was performed in the six patients enrolled in the first step of the study. Venous blood samples were taken prior to erlotinib dosing on day 3 and day 8 of cycle 1 at 0.5, 1, 2, 4, 6, 8 and 24 h after erlotinib administration. Samples were also taken prior to gemcitabine infusion on days 1 and 8 at 0.5, 0.75, 1, 1.5, 2.5 and 4.5 h after dosing.

The plasma concentrations of erlotinib, OSI-420 and gemcitabine were measured by liquid chromatography, tandem mass spectrometry (LC-MS-MS). The LC-MS-MS analytical methods have been described previously.[31,32] Derived PK parameters included the maximum plasma drug concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma drug concentration-time curve to the last plasma sample (AUC_{last}), terminal half-life ($t_{1/2}$) and oral clearance (Cl/F).

Biomarker analysis. *EGFR* mutations were assessed in patients with available tumor tissue specimens, which were formalin fixed and paraffin embedded. Samples were analyzed at a central laboratory where DNA was extracted and exons 18–21 sequenced using a nested PCR.

Statistical analysis. Progression-free survival and OS were estimated using the Kaplan–Meier method in all patients who received at least one dose of the study treatment, with 95% CI for the median duration calculated using Greenwood's formula. The Clopper–Pearson method was used to calculate the 95% CI around the ORR, DCR and AE rate. Multivariate analyses were performed for the occurrence of ILD-like events using the logistic regression model. Baseline characteristics investigated for this analysis included gender, age, lung metastasis, emphysema and various baseline laboratory values. The target enrollment was 100 patients, as this was required to evaluate the safety of erlotinib.

Results

Patient characteristics. Between December 2006 and October 2007, a total of 107 patients were enrolled (first step, n = 6; second step, n = 101) from 12 institutions (Fig. 1). One patient who enrolled into the second step did not receive treatment due to deterioration in PS prior to the start of treatment. A total of 106 patients were evaluable for safety (safety population, full analysis set).

The patient demographics and baseline characteristics are shown in Table 1. The median age was 62 years (range, 36–78) and 52.8% of patients were male. Almost all patients were chemonaïve (95.3%). The majority (75.5%) of patients had an ECOG PS of 0 and most (83.0%) had metastatic disease. Over half (63.2%) of the patients had a history of current or past smoking.

Toxicity and dose modifications. The median duration of erlotinib exposure was 102.5 days and its median dose intensity was 100.0 mg/day, with the majority of patients (78.3%) receiving more than 90% of the relative dose intensity. The median duration of gemcitabine treatment was 4.0 cycles and its median dose intensity was 688.0 mg/m^2 per week, with approximately half of the patients (51.4%) receiving more than 90% of the relative dose intensity.

As only one patient had a DLT (grade 3 diarrhea) in the first step, the second step of the study was initiated. One hundred and six patients received at least one dose of erlotinib; these patients were assessable for toxicity. Treatment-related AE and treatment-related changes in laboratory values are summarized in Table 2; most of these were mild to moderate in severity. The most frequently reported AE was RASH, which occurred in

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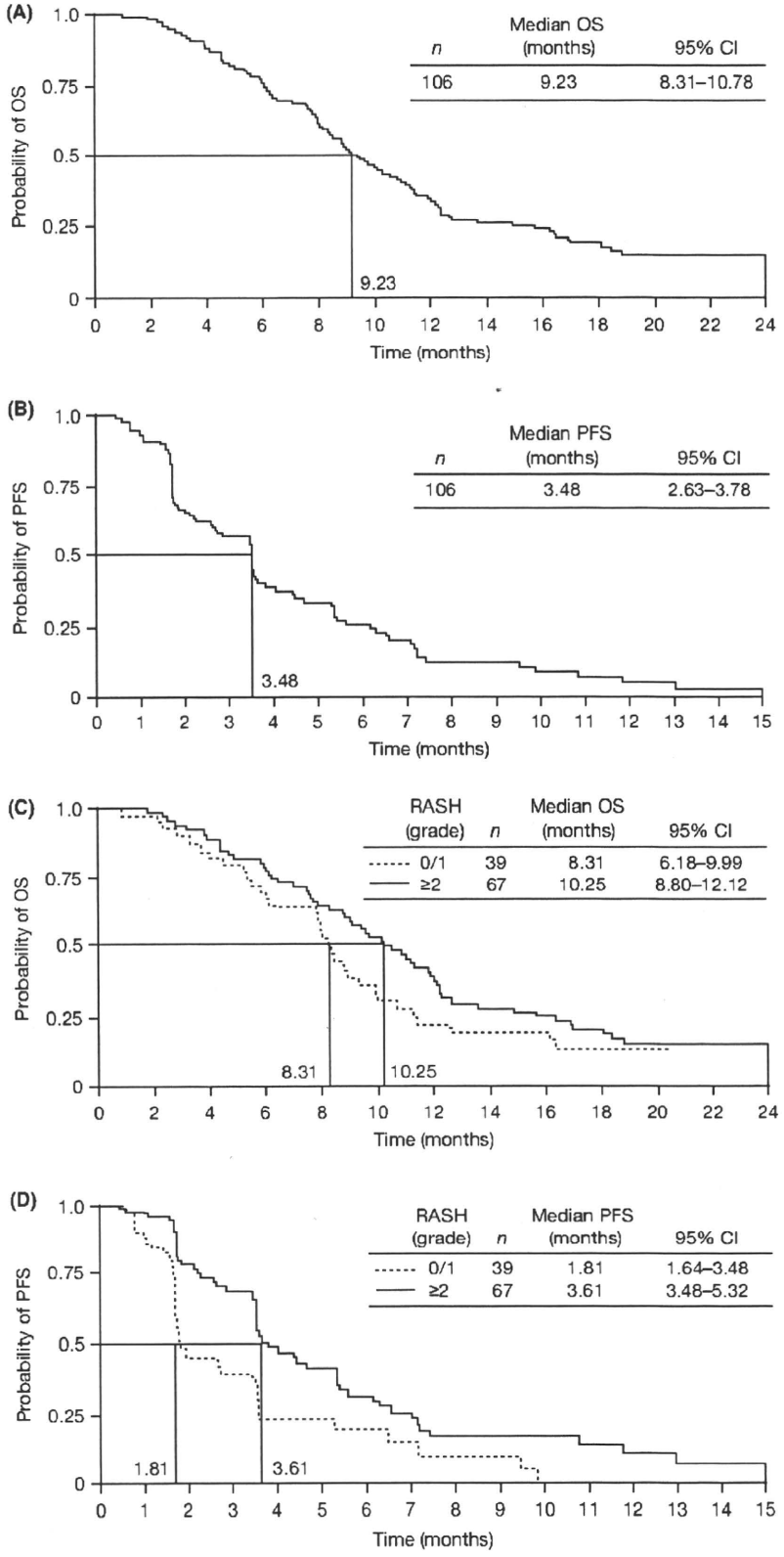

Fig. 1. Kaplan–Meier estimates of (A) overall survival (OS) and (B) progression-free survival (PFS) in the study population ($n = 106$); (C) OS and (D) PFS according to the severity of RASH (grade ≤1 [$n = 39$] *vs* grade ≥2 [$n = 67$]). RASH is a composite of the terms: rash, acne, exfoliative rash, dermatitis acneiform, erythema, eczema, dermatitis and pustular rash. CI, confidence interval.

Table 1. Baseline characteristics and demographics (n = 106)

Characteristic	
Median age (range) (years)	62 (36–78)
Gender, n (%)	
Male	56 (52.8)
Female	50 (47.2)
Median bodyweight (range) (kg)	52.3 (33.1–95.0)
Smoking history,† n (%)	
Never smoker	39 (36.8)
Past smoker	37 (34.9)
Current smoker	30 (28.3)
ECOG PS, n (%)	
0	80 (75.5)
1	26 (24.5)
2	0 (0)
Disease status, n (%)	
Metastatic	88 (83.0)
Locally advanced	18 (17.0)
Primary tumor identified, n (%)	92 (86.8)
Primary sites, n (%)	
Head	46 (43.4)
Body and tail	23 (21.7)
Body	22 (20.8)
Tail	10 (9.4)
Other	5 (4.7)‡
Biliary drainage, n (%)	19 (17.9)
Sites of distant metastases, n (%)	
Liver	56 (52.8)
Distant lymph nodes	39 (36.8)
Lung	17 (16.0)
Other	26 (24.5)
Prior lines of therapy, n (%)	
None	101 (95.3)
One regimen	5 (4.7)§
Median CA19–9 (range) (U/mL)	
Median	776 (0–435 000)
Median CEA (range) (ng/mL)	
Median	4.8 (0.6–1100.1)

†Never smoker, never/hardly smoked; past smoker, passage of at least 1 month since stopping smoking (at the time of registration); current smoker, smoked within 1 month (at the time of registration). ‡Whole of pancreas (n = 1); head and body (n = 3); other (n = 1). §Tegafur, gimeracil, oteracil potassium (S-1) (n = 3); 5-fluorouracil plus leucovorin (n = 2). CA 19–9, carbohydrate antigen 19–9; CEA, carcinoembryonic antigen; ECOG, Eastern Co-Operative Group.

93.4% of the patients; most cases were mild to moderate in severity (87.7%, grade ≤2; 5.7%, grade ≥3). Other common non-hematological AE included anorexia, pruritus, fatigue, nausea and diarrhea. Most patients experienced some degree of hematological toxicity, with grade 3 or 4 neutropenia (neutrophil decreased), leucopenia (white blood cell count decreased) and anemia (hemoglobin decreased) occurring in 34.9%, 29.2% and 14.2% of patients, respectively. Only one treatment-related death occurred (due to gastrointestinal hemorrhage), which was probably due to arterial bleeding caused by the invasion of the primary tumor into the gastrointestinal tract. Although the likelihood of this event being treatment-related was deemed remote, a causal relationship could not be completely excluded because the event occurred during the study treatment administration period.

Treatment-related SAE were reported in 26 (24.5%) patients. These included nine ILD-like events (8.5%), the majority of which (n = 7) were grade 1–2 in severity. Importantly, all of these nine patients recovered or improved, and four of these patients did so without any treatment for ILD-like events. Other

Table 2. Treatment-related adverse events occurring in >30% of patients treated with erlotinib and gemcitabine (n = 106)

	Any grade, n (%)	Grade 3, n (%)	Grade 4, n (%)
Non-hematological			
Rash	78 (73.6)	3 (2.8)	0 (0)
Anorexia	75 (70.8)	15 (14.2)	0 (0)
Pruritus	57 (53.8)	1 (0.9)	0 (0)
Fatigue	56 (52.8)	3 (2.8)	0 (0)
Nausea	56 (52.8)	6 (5.7)	0 (0)
Diarrhea	52 (49.1)	2 (1.9)	0 (0)
Dry skin	49 (46.2)	0 (0)	0 (0)
Stomatitis	38 (35.8)	0 (0)	0 (0)
Pyrexia	32 (30.2)	0 (0)	0 (0)
Hematological			
White blood cell count decreased	85 (80.2)	31 (29.2)	0 (0)
Platelet count decreased	77 (72.6)	9 (8.5)	0 (0)
Hemoglobin decreased	76 (71.7)	13 (12.3)	2 (1.9)
Hematocrit decreased	73 (68.9)	8 (7.5)	0 (0)
Neutrophil decreased	73 (68.9)	32 (30.2)	5 (4.7)
Red blood cell count decreased	72 (67.9)	8 (7.5)	0 (0)
ALT increased	59 (55.7)	10 (9.4)	0 (0)
AST increased	57 (53.8)	4 (3.8)	1 (0.9)
Weight decreased	53 (50.0)	3 (2.8)	0 (0)
Lymphocyte count decreased	46 (43.4)	14 (13.2)	0 (0)
Blood albumin decreased	35 (33.0)	0 (0)	0 (0)
Gamma-glutamyltransferase increased	35 (33.0)	12 (11.3)	1 (0.9)

ALT, alanine amino transferase; AST, aspartate amino transferase.

treatment-related SAE were anorexia (3.8%), vomiting, pyrexia and abnormal hepatic function (1.9% each). The baseline characteristics, treatment and outcomes of patients who developed treatment-related ILD-like events during the study are detailed in Table 3. The onset times of ILD-like events ranged from 7 to 187 days after the start of treatment. In these patients, a relatively long survival was observed (from 119 to 568+ days), and five patients received post-study therapy. All of these nine patients were past or current smokers, and six had emphysema at baseline (not detected prior to treatment, but diagnosed at the extramural review by a radiologist in the DSMC). Multivariate analyses were performed for the occurrence of ILD-like events using the logistic regression model and emphysema at baseline was indicated as a risk factor for onset of ILD-like events (odds ratio [95% CI], 12.13 [1.01–145.7]; P = 0.0491).

Adverse events led to erlotinib discontinuation in 30 patients (28.3%) and gemcitabine discontinuation in 27 patients (25.5%). The main reasons for treatment discontinuation were ILD (n = 6) and anorexia (n = 3); no patient discontinued treatment due to RASH or diarrhea. Due to the onset of AE, a total of 65 patients (61.3%) required one or more interruptions of erlotinib (36 patients [34.0%] for longer than seven consecutive days and 17 patients [16.0%] for longer than 14 consecutive days) and 56 patients (52.8%) had one or more skip of gemcitabine. Modifications in the erlotinib or gemcitabine dosage were required in 17 (16.0%) and 11 (10.4%) patients, respectively, due to AE.

Efficacy. The median OS was 9.23 months (95% CI, 8.31–10.78; Fig. 1A) and the 1-year survival rate was 33% (95% CI, 24–42). Median PFS was 3.48 months (95% CI, 2.63–3.78; Fig. 1B). Among the patients evaluable for tumor response (n = 64), the ORR was 20.3% (13/64; 95% CI, 11.3–32.2) and the DCR was 50.0% (95% CI, 37.2–62.8; CR, n = 0; PR, n = 13; stable disease, n = 19).

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