approximates the annual incidence, thereby reflecting a generally short survival time associated with pancreatic cancer, which is generally less than 1 year. Cancer of the pancreas has the shortest median survival time out of all cancer types in a stage for stage basis. Early diagnosis is the most important factor for improving the overall survival and quality of life in patients with pancreatic cancer.

Recently, positron emission tomography (PET) has demonstrated superiority to computed tomography (CT), ultrasonography (US), and endoscopic US (EUS) in its sensitivity and specificity in diagnosing pancreatic cancer[3-6]. Furthermore, the metabolic activity of the tumor may be of prognostic significance. We have been reported the efficacy of delayed additional ^{18}F-fluorodeoxyglucose PET (FDG-PET) imaging in the differential diagnosis of malignant from benign lesions in patients who are suspected of having pancreatic cancer[7]. Furthermore, the detection rate of liver metastases smaller than 1 cm in diameter from pancreatic cancer was only 33% on early image and 58% on delayed image[7]. However, the role of dual time point FDG-PET in the diagnosis of small pancreatic cancers has yet to be established.

Therefore, the present study investigated whether small cancers of the pancreas could be accurately diagnosed by FDG-PET with dual time point evaluation.

MATERIALS AND METHODS

Patients

Thirty-one patients with pancreatic carcinoma suspected on the basis of conventional radiological studies (22 males and 9 females; mean age, 65 years; age range, 44-82 years) and who underwent FDG-PET between 2003 and 2007 were retrospectively selected. Patients were excluded from this study if they had poorly controlled diabetes mellitus (presenting with blood glucose level > 200 mg/dL prior to PET imaging). Conventional radiological staging was performed by means of CT or magnetic resonance imaging (MRI). The location of the cancer was in the head of the pancreas in 17 patients and in the body and tail in 14 patients. Twelve of the 31 cancers were diagnosed to be unresectable, and 19 patients eventually underwent surgery with a curative intention, although the cancer turned out to be unresectable in 7 because of intraoperative findings.

Methods

The patients were divided into 3 groups according to the maximum diameter of the tumor: TS1 (maximum size ≤ 2.0 cm), TS2 (> 2.0 cm and ≤ 4.0 cm) or TS3-4 (> 4.0 cm) as indicated by the classification system of the Japan Pancreas Society. FDG-PET was analyzed semi-quantitatively using the standardized uptake values (SUVs). The sensitivity of diagnosing pancreatic cancer was examined for FDG-PET, CT, MRI and the serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) with regard to the size of the tumor. The details of SUVs, the histological findings and correlation of CT findings were evaluated in patients with TS1 pan-

creatic cancer. This study was performed retrospectively by collecting and analyzing data from the patient records.

FDG-PET

The FDG-PET images were acquired with a PET machine (Siemens EXACT HR+, CTI, Knoxville, TN, USA). The patients were required to fast for at least 4 h before PET imaging. The emission images were acquired (early image) 1 h after the intravenous administration of 5 mCi of FDG. Delayed PET emission images of the upper abdomen were acquired at 2 h after administration of ^{18}F-FDG, using 2 or 3 bed positions with a 3-min acquisition at each[7]. This acquisition was immediately followed by a transmission scan of the same transverse planes, using a 2-min acquisition at each bed position. The early and delayed PET images were reviewed independently and consecutively by 2 radiologists with extensive experience in FDG-PET imaging. PET images were compared with the corresponding CT and/or MRI images for accurate anatomical identification of the tumor. The findings were considered to be positive when both radiologists strongly suspected malignant disease. In addition, the images were analyzed semi-quantitatively using the SUV, as reported elsewhere. Briefly, for semi-quantitative analysis, a region of interest was placed over the entire FDG-avid lesion including the largest amount of radioactivity using the transverse PET image. The SUV was calculated as: SUV = (activity in region of interest in mCi)/(injected dose in mCi/weight in kg).

CT

CT studies were performed with a multidetector row CT scanner (Aquilion, Toshiba, Tokyo, Japan). Helical images of the abdomen were routinely obtained and reconstructed with 5 mm thickness. After pre-contrast CT scans, arterial dominant phase images of dynamic CT were obtained starting 40 s after the beginning of the intravenous bolus injection (3 mL/s) of 100 mL of iodized contrast medium at 350 mg/mL. The pancreatic phase and the late phase (near equilibrium phase) were also obtained, starting at 60 and 180 s after injection, respectively. The CT images were interpreted independently and consecutively by 2 radiologists with extensive experience of more than 10 years in CT scanning. The findings of the CT scans were considered positive when both radiologists strongly suspected malignant disease due to a discrete low-attenuation mass within the pancreas.

MRI

Two 1.5 T superconducting units, Signa Advantage (General Electric, Milwaukee, WI, USA, USA) and Visart (Toshiba, Tokyo, Japan), were used for MRI. T1-weighted gradient-echo imaging; FS-T2-weighted turbo SE imaging and heavily T2-weighted turbo SE images were acquired in the order of scan after initial T1-weighted localizing images were obtained in the coronal and trans-axial directions.

Statistical analysis

The χ^2 test was employed for a statistical comparison of

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 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.

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.
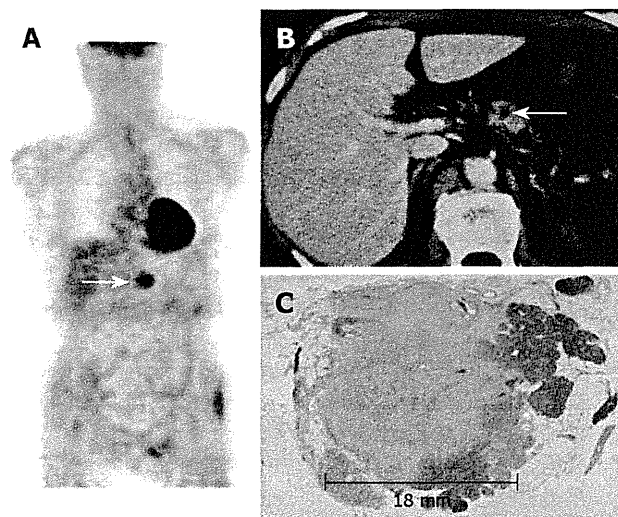

Figure 1 Positron emission tomography images of a 68-year-old male with TS1 pancreatic cancer. A: Whole body positron emission tomography image shows apparent increased uptake of ^{18}F-fluorodeoxyglucose in the tumor (arrow, delayed point standardized uptake value, 3.06); B: Axial computed tomography 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 iden-

tified 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 18F-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.

REFERENCES

1 Matsuno S, Egawa S, Arai K. Trends in treatment for pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2001; **8**: 544-548

2 Yamamoto M, Ohashi O, Saitoh Y. Japan Pancreatic Cancer Registry: current status. *Pancreas* 1998; **16**: 238-242

3 Wakabayashi H, Nishiyama Y, Otani T, Sano T, Yachida S, Okano K, Izuishi K, Suzuki Y. Role of 18F-fluorodeoxyglucose positron emission tomography imaging in surgery for pancreatic cancer. *World J Gastroenterol* 2008; **14**: 64-69

4 Pakzad F, Groves AM, Ell PJ. The role of positron emission tomography in the management of pancreatic cancer. *Semin Nucl Med* 2006; **36**: 248-256

5 Hanbidge AE. Cancer of the pancreas: the best image for early detection--CT, MRI, PET or US? *Can J Gastroenterol* 2002; **16**: 101-105

6 Sato M, Okumura T, Kaito K, Kiyoshima M, Asato Y, Uchiumi K, Iijima H, Hashimoto I, Kaburagi T, Amemiya R. Usefulness of FDG-PET/CT in the detection of pancreatic metastases from lung cancer. *Ann Nucl Med* 2009; **23**: 49-57

7 Nishiyama Y, Yamamoto Y, Monden T, Sasakawa Y, Tsutsui K, Wakabayashi H, Ohkawa M. Evaluation of delayed additional FDG PET imaging in patients with pancreatic tumour. *Nucl Med Commun* 2005; **26**: 895-901

8 Ichikawa T, Haradome H, Hachiya J, Nitatori T, Ohtomo K, Kinoshita T, Araki T. Pancreatic ductal adenocarcinoma: preoperative assessment with helical CT versus dynamic MR imaging. *Radiology* 1997; **202**: 655-662

9 Sheridan MB, Ward J, Guthrie JA, Spencer JA, Craven CM, Wilson D, Guillou PJ, Robinson PJ. Dynamic contrast-enhanced MR imaging and dual-phase helical CT in the preoperative assessment of suspected pancreatic cancer: a comparative study with receiver operating characteristic analysis. *AJR Am J Roentgenol* 1999; **173**: 583-590

10 Legmann P, Vignaux O, Dousset B, Baraza AJ, Palazzo L, Dumontier I, Coste J, Louvel A, Roseau G, Couturier D, Bonnin A. Pancreatic tumors: comparison of dual-phase helical CT and endoscopic sonography. *AJR Am J Roentgenol* 1998; **170**: 1315-1322

11 Tabuchi T, Itoh K, Ohshio G, Kojima N, Maetani Y, Shibata T, Konishi J. Tumor staging of pancreatic adenocarcinoma using early- and late-phase helical CT. *AJR Am J Roentgenol* 1999; **173**: 375-380

12 Kaneko K, Honda H, Hayashi T, Fukuya T, Ro T, Irie H, Masuda K. Helical CT evaluation of arterial invasion in pancreatic tumors: comparison with angiography. *Abdom Imaging* 1997; **22**: 204-207

13 DeWitt J, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004; **141**: 753-763

14 Erturk SM, Ichikawa T, Motosugi U, Sou H, Araki T. Diffusion-weighted MR imaging in the evaluation of pancreatic exocrine function before and after secretin stimulation. *Am J Gastroenterol* 2006; **101**: 133-136

15 Seo S, Doi R, Machimoto T, Kami K, Masui T, Hatano E, Ogawa K, Higashi T, Uemoto S. Contribution of 18F-fluorodeoxyglucose positron emission tomography to the diagnosis of early pancreatic carcinoma. *J Hepatobiliary Pancreat Surg* 2008; **15**: 634-639

16 Sperti C, Pasquali C, Chierichetti F, Ferronato A, Decet G, Pedrazzoli S. 18-Fluorodeoxyglucose positron emission tomography in predicting survival of patients with pancreatic carcinoma. *J Gastrointest Surg* 2003; **7**: 953-959; discussion 959-960

17 Maemura K, Takao S, Shinchi H, Noma H, Mataki Y, Kurahara H, Jinnouchi S, Aikou T. Role of positron emission tomography in decisions on treatment strategies for pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2006; **13**: 435-441

18 Chowdhury P, Rayford PL. Smoking and pancreatic disorders. *Eur J Gastroenterol Hepatol* 2000; **12**: 869-877

19 Lowenfels AB, Maisonneuve P. Epidemiologic and etiologic factors of pancreatic cancer. *Hematol Oncol Clin North Am* 2002; **16**: 1-16

20 Simon B, Printz H. Epidemiological trends in pancreatic neoplasias. *Dig Dis* 2001; **19**: 6-14

21 Ghadirian P, Lynch HT, Krewski D. Epidemiology of pancreatic cancer: an overview. *Cancer Detect Prev* 2003; **27**: 87-93

22 Nakamoto Y, Higashi T, Sakahara H, Tamaki N, Kogire M, Doi R, Hosotani R, Imamura M, Konishi J. Delayed (18)F-fluoro-2-deoxy-D-glucose positron emission tomography scan for differentiation between malignant and benign lesions in the pancreas. *Cancer* 2000; **89**: 2547-2554

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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 population. Lifestyle related diseases, such as malignant neoplasms, cardiovascular diseases and cerebrovascular diseases, are becoming more prevalent as a cause of death. At present, malig-

nant 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 both 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.
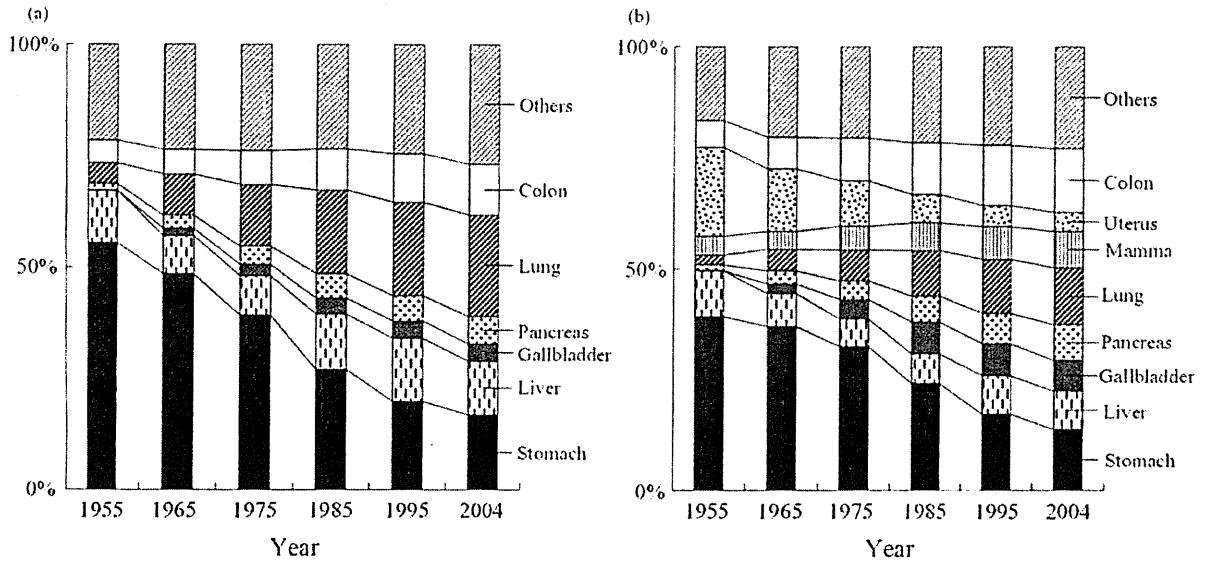
Figure 1 Changes in cause of death by malignant neoplasm since 1955. Data are derived from: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suii05/deth15.html. (a) Male; (b) Female.
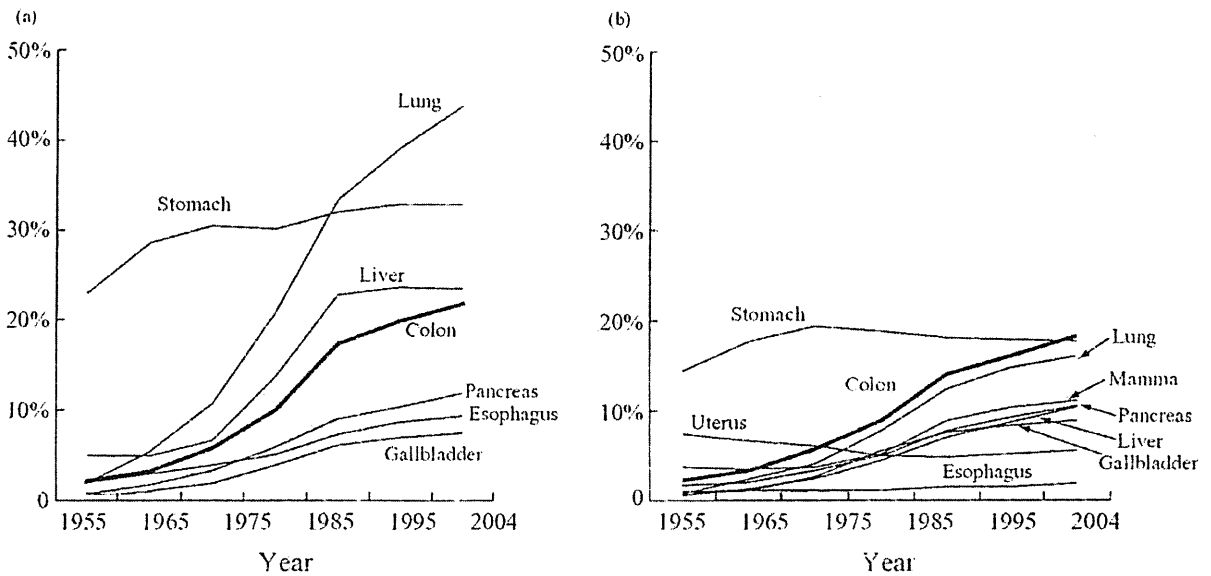
Figure 2 Percent changes in cause of death by malignant neoplasm. Data are derived from: http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/suii05/deth16.html (a) Male; (b) Female.

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).[3–6]

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 subject 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.[im-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.

References

1 Achuller HM. Mechanisms of smoking-related lung and pancreatic adenocarcinoma development. *Nature Rev Cancer* 2002; 2: 255–263.

2 Ezzati M, Rodgers A, Lopez AD *et al.* Mortality and burden of disease attributable to individual risk factors. In: Ezzati M, Lopez AD, Rodgers A, Murray CJL eds. *Comparative quantification of health risks. Global and regional burden of disease attributable to selected major risk factors.* Geneva: World Health Organization, 2004; 2: 2141–2166.

3 Boeing H. Alcohol and risk of cancer of the upper gastrointestinal tract: first analysis of the EPIC data. In: Riboli E, Lambert R, eds. *Nutrition and lifestyle: Opportunities for cancer prevention.* SARC Sci Publ 156. Lyon: International Agency for Research on Cancer, 2002; 151–154.

4 Talamini R. Bosetti C, La Vecchia C *et al.* Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. *Cancer Causes Control* 2002; 13: 957–964.

5 Znaor A, Brennan P, Gajalakshmi V *et al.* Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. *Int J Cancer* 2003; 105: 681–686.

6 Muto M, Nakane M, Hitomi Y *et al.* Association between aldehyde dehydrogenase gene polymorphisms and the phenomenon of field cancerization in patients with head and neck cancer. *Carcinogenesis* 2002; 23: 1759–1765.

7 Higuchi S, Matsushita S, Murayama M *et al.* Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. *Am J Psychiatry* 1995; 152: 1219–1221.

8 Yokoyama A, Omori T. Genetic polymorphisms of alcohol and aldehyde dehydrogenases and risk for esophageal and head and neck cancers. *Alcohol* 2005; 35: 175–185.

9 Lewis SJ, Smith GD. Alcohol, ALDH2, and esophageal cancer: a meta-analysis which illustrates the potentials and limitations of a Mendelian randomization approach. *Cancer Epidemiol Biomakers Prev* 2005; 14: 1967–1971.

10 Matsuda T, Yabushita H, Kanaly RA *et al.* Increased DNA damage in ALDH2-deficient alcoholics. *Chem Res Toxicol* 2006; 19: 1374–1378.

11 Miyasaka K, Kawanami T, Shimokata H *et al.* Inactive aldehyde dehydrogenase-w increased the risk of pancreatic cancer among smokers in a Japanese male population. *Pancreas* 2005; 30: 95–98.

12 Watch P. Carcinogenicity of alcoholic beverages. *Lancet Oncology* 2007; 8: 292–293.

13 Bagnardi V, Blangiardo M, La Vecchia C *et al.* A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer* 2001; 85: 1700–1705.

14 Shimokata H, Yamada Y, Nakagawa M *et al.* Distribution of geriatric disease-related genotypes in the National Institute of Longevity Sciences, Longitudinal Study of Aging (NILS-LSA). *J Epidemiol* 2000; 10 (suppl): S46–S5.

15 Kamino K, Nagasaka K, Imagawa M *et al.* Deficiency in mitochondrial aldehyde dehydrogenase increases the risk for late-onset Alzheimer's in the Japanese population. *Biochem Biophys Res Comm* 2000; 273: 192–196.

16 Matsuno S, Egawa S, Shibuya K *et al.* Pancreatic cancer: current status of treatment and survival of 16071 patients diagnosed from 1981–1996, using Japanese national pancreatic cancer database. *Int J Clin Oncol* 2000; 5: 153–157.

17 Karlson BM, Ekbom A, Josefsson S *et al.* The risk of pancreatic cancer following pancreatitis: An association due to confounding? *Gastroenterology* 1997; 113: 587–592.

18 Ye W, Lagergren J, Weiderpass E *et al.* Alcohol abuse and the risk of pancreatic cancer. *Gut* 2002; 51: 236–239.

19 Lin Y, Tamakoshi A, Matsuno S *et al.* Nationwide epidemiological survey of CP in Japan. *J Gastroenterol* 2000; 35: 135–141.

20 Lowenfels AB, Maisonneuve P, Cavallini G *et al.* Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; 328: 1433–1437.

21 Bansal P, Sonnenberg A. Pancreatitis is a risk factor for pancreatic cancer. *Gastroenterology* 1995; 109: 247–252.

22 Howes N, Neoptolemos JP. Risk of pancreatic ductal adenocarcinoma in chronic pancreatitis. *Gut* 2002; 51: 765–766.

23 Malka D, Hammel P, Maire F *et al.* Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut* 2002; 51: 849–852.

24 Yokoyama A, Muramatsu T, Ohmori T *et al.* Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. *Cancer* 1996; 77: 1986–1990.

25 Woutersen RA, Appelman LM, Van Garderen-Hoetmer A *et al.* Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicol* 1986; 41: 213–231.

26 Harada S, Agarwal DP, Goedde HW. Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. *Lancet* 1981; ii: 982.

27 Calle EE, Rodriguez C, Walker-Thurmond K *et al.* Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. Adults. *N Engl J Med* 2003; 348: 1925–1938.

28 Bianchini F, Kaaks R, Vainio H. Overweight, obesity and cancer risk. *Lancet Oncol* 2002; 3: 565–574.

29 Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; 4: 579–591.

30 Terry PD, Miller AB, Rohan TE. Obesity and colorectal cancer risk in women. *Gut* 2002; 51: 191–194.

31 Giovannucci E, Ascherio A, Rimm EB *et al.* Physical activity, obesity, and risk for colon cancer and adenoma in men. *Ann Intern Med* 1995; 122: 327–334.

32 Terry P, Giovannucci E, Bergkvist L *et al.* Body weight and colorectal cancer risk in a cohort of Swedish women: relation varies by age and cancer site. *Br J Cancer* 2001; 85: 346–349.

33 Kaaks R, Toniolo P, Akhmedkhanov A *et al.* Serum C-peptide, insulin-like growth factor (IGF)-1, IGF-binding proteins, and colorectal cancer risk in women. *J Natl Cancer Inst* 2000; 92: 1592–1600.

34 Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. *Gastroenterology* 2004; 127: 1044–1050.

35 Chiu HM, Lin JT, Shun CT *et al.* Association of metabolic syndrome with proximal and syndchronous colorectal neoplasm. *Clinical Gastroenterology and Hepatology* 2007; 5: 221–229.

36 Cho E, Smith-Warner Sa, Ritz J *et al.* Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med* 2004; 140: 603–613.

37 Moskal A, Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies. *Int J Cancer* 2007; 120: 664–671.

38 World Cancer Research fund and American Institute of Cancer Research: Food, nutrition and prevention of cancer: A global perspective. American Institute for Cancer research. Washington DC. 1997.

39 World Health Organization/Food and Agriculture Organization of the United Nations Food: Diet, nutrition and the prevention of chronic diseases, report of a joint WHO/FAO expert consultation. Geneva: WHO Technical Report Series 2003; 916.

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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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.

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,
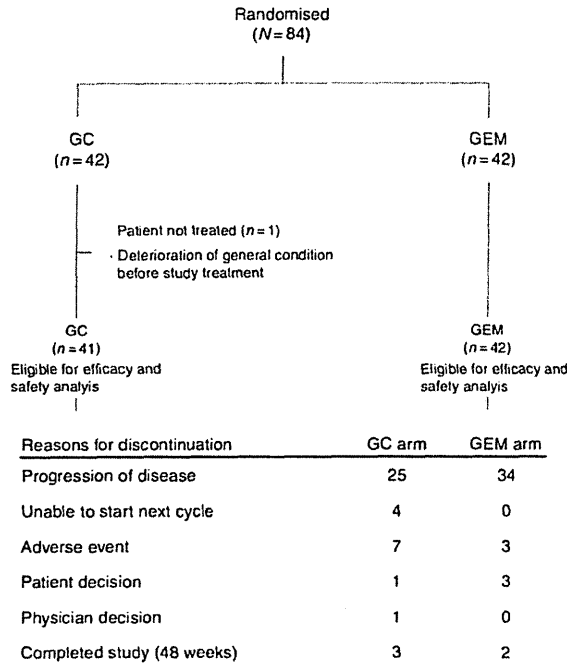
Randomised
(N = 84)

GC
(n = 42)

GEM
(n = 42)

Patient not treated (n = 1)
· Deterioration of general condition
before study treatment

GC
(n = 41)
Eligible for efficacy and
safety analyis

GEM
(n = 42)
Eligible for efficacy and
safety analyis

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)[b]	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).

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
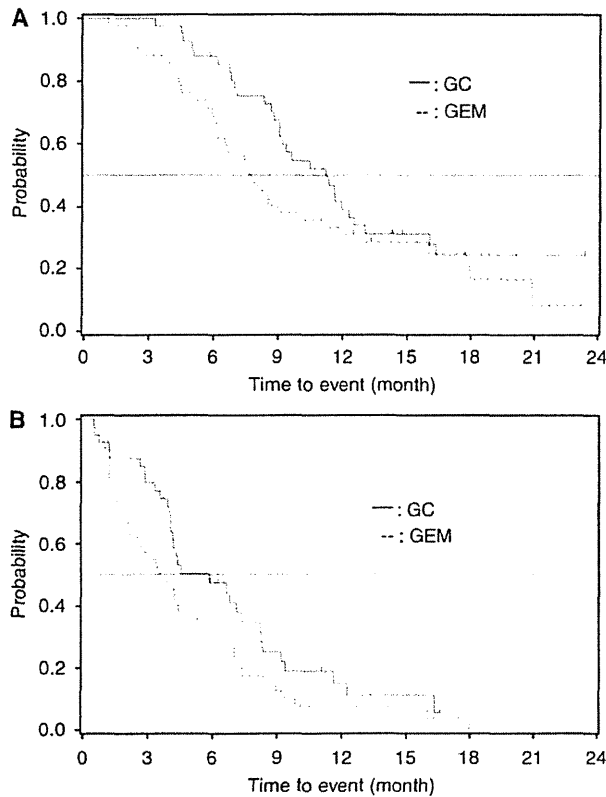

Figure 2 Kaplan–Meier curve of overall survival and progression-free survival. (**A**) Overall survival. (**B**) Progression-free survival. GC = gemcitabine–cisplatin combination; GEM = gemcitabine alone; CI = confidence interval.

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.

REFERENCES

Aljiffry M, Walsh MJ, Molinari M (2009) Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990-2009. *World J Gastroenterol* **15:** 4240–4262

Bergman AM, Ruiz van Haperen VW, Veerman G, Kuiper CM, Peters GJ (1996) Synergistic interaction between cisplatin and gemcitabine *in vitro*. *Clin Cancer Res* **2:** 521–530

Charoentum C, Thongprasert S, Chewaskulyong B, Munprakan S (2007) Experience with gemcitabine and cisplatin in the therapy of inoperable and metastatic cholangiocarcinoma. *World J Gastroenterol* **13:** 2852–2854

Doval DC, Sekhon JS, Gupta SK, Fuloria J, Shukla VK, Gupta S, Awasthy BS (2004) A phase II study of gemcitabine and cisplatin in chemotherapy-naive, unresectable gall bladder cancer. *Br J Cancer* **90:** 1516–1520

Eckel F, Schmid RM (2007) Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* **96:** 896–902

Goodman MT, Yamamoto J (2007) Descriptive study of gallbladder, extrahepatic bile duct, and ampullary cancers in the United States, 1997–2002. *Cancer Causes Control* **18:** 415–422

Jarnagin WR, Klimstra DS, Hezel M, Gonen M, Fong Y, Roggin K, Cymes K, DeMatteo RP, D'Angelica M, Blumgart LH, Singh B (2006) Differential cell cycle-regulatory protein expression in biliary tract adenocarcinoma: correlation with anatomic site, pathologic variables, and clinical outcome. *J Clin Oncol* **24:** 1152–1160

Kim ST, Park JO, Lee J, Lee KT, Lee JK, Choi SH, Heo JS, Park YS, Kang WK, Park K (2006) A Phase II study of gemcitabine and cisplatin in advanced biliary tract cancer. *Cancer* **106:** 1339–1346

Lee J, Kim TY, Lee MA, Ahn MJ, Kim HK, Lim HY, Lee NS, Park BJ, Kim JS (2008) Phase II trial of gemcitabine combined with cisplatin in patients with inoperable biliary tract carcinomas. *Cancer Chemother Pharmacol* **61:** 47–52

Matsuda T, Marugame T (2007) International comparisons of cumulative risk of gallbladder cancer and other biliary tract cancer, from Cancer Incidence in Five Continents Vol. VIII *Jpn J Clin Oncol* **37:** 74–75

Meyerhardt JA, Zhu AX, Stuart K, Ryan DP, Blaszkowsky L, Lehman N, Earle CC, Kulke MH, Bhargava P, Fuchs CS (2008) Phase-II study of gemcitabine and cisplatin in patients with metastatic biliary and gallbladder cancer. *Dig Dis Sci* **53:** 564–570

Okusaka T, Ishii H, Funakoshi A, Yamao K, Ohkawa S, Saito S, Saito H, Tsuyuguchi T (2006) Phase II study of single-agent gemcitabine in patients with advanced biliary tract cancer. *Cancer Chemother Pharmacol* **57:** 647–653

Park BK, Kim YJ, Park JY, Bang S, Park SW, Chung JB, Kim KS, Choi JS, Lee WJ, Song SY (2006) Phase II study of gemcitabine and

cisplatin in advanced biliary tract cancer. *J Gastroenterol Hepatol* **21:** 999–1003

Park I, Lee JL, Ryu MH, Kim TW, Sook Lee S, Hyun Park D, Soo Lee S, Wan Seo D, Koo Lee S, Kim MH (2009) Prognostic factors and predictive model in patients with advanced biliary tract adenocarcinoma receiving first-line palliative chemotherapy. *Cancer* **115:** 4148–4155

Pasetto LM, D'Andrea MR, Falci C, Monfardini S (2007) Gemcitabine in advanced biliary tract cancers. *Crit Rev in Oncol Hematol* **61:** 230–242

Peters GJ, Bergman AM, Ruiz van Haperen VW, Veerman G, Kuiper CM, Braakhuis BJ (1995) Interaction between cisplatin and gemcitabine *in vitro* and *in vivo*. *Semin Oncol* **22**(4 Suppl 11): 72–79

Randi G, Malvezzi M, Levi F, Ferlay J, Negri E, Franceschi S, La Vecchia C (2009) Epidemiology of biliary tract cancers: an update. *Ann Oncol* **20:** 146–159

Simon R, Wittes RE, Ellenberg SS (1985) Randomized phase II clinical trials. *Cancer Treat Rep* **69:** 1375–1381

Thongprasert S, Napapan S, Charoentum C, Moonprakan S (2005) Phase II study of gemcitabine and cisplatin as first-line chemotherapy in inoperable biliary tract carcinoma. *Ann Oncol* **16:** 279–281

Valle JW, Wasan H, Johnson P, Jones E, Dixon L, Swindell R, Baka S, Maraveyas A, Corrie P, Falk S, Gollins S, Lofts F, Evans L, Meyer T, Anthoney A, Iveson T, Highley M, Osborne R, Bridgewater J (2009a) Gemcitabine alone or in combination with cisplatin in patients with advanced or metastatic cholangiocarcinomas or other biliary tract tumours: a multicentre randomised phase II study - The UK ABC-01 Study. *Br J Cancer* **101:** 621–627

Valle JW, Wasan HS, Palmer DD, Cunningham D, Anthoney DA, Maraveyas A, Hughes SK, Roughton M, Bridgewater JA (2009b) Gemcitabine with or without cisplatin in patients with advanced or metastatic biliary tract cancer (ABC): Results of a multicenter, randomized phase III trial (the UK ABC-02 trial). *J Clin Oncol* **27:** 15s (Suppl; abstract 4503)(Recently the results of this study were published as follows: Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J; ABC-02 Trial Investigators (2010) Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* **362:** 1273–1281)

Wagner AD, Buechner-Steudel P, Moehler M, Schmalenberg H, Behrens R, Fahlke J, Wein A, Behl S, Kuss O, Kleber G, Fleig WE (2009) Gemcitabine, oxaliplatin and 5-FU in advanced bile duct and gallbladder carcinoma: two parallel, multicentre phase-II trials. *Br J Cancer* **101:** 1846–1852

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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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 pre-specified 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
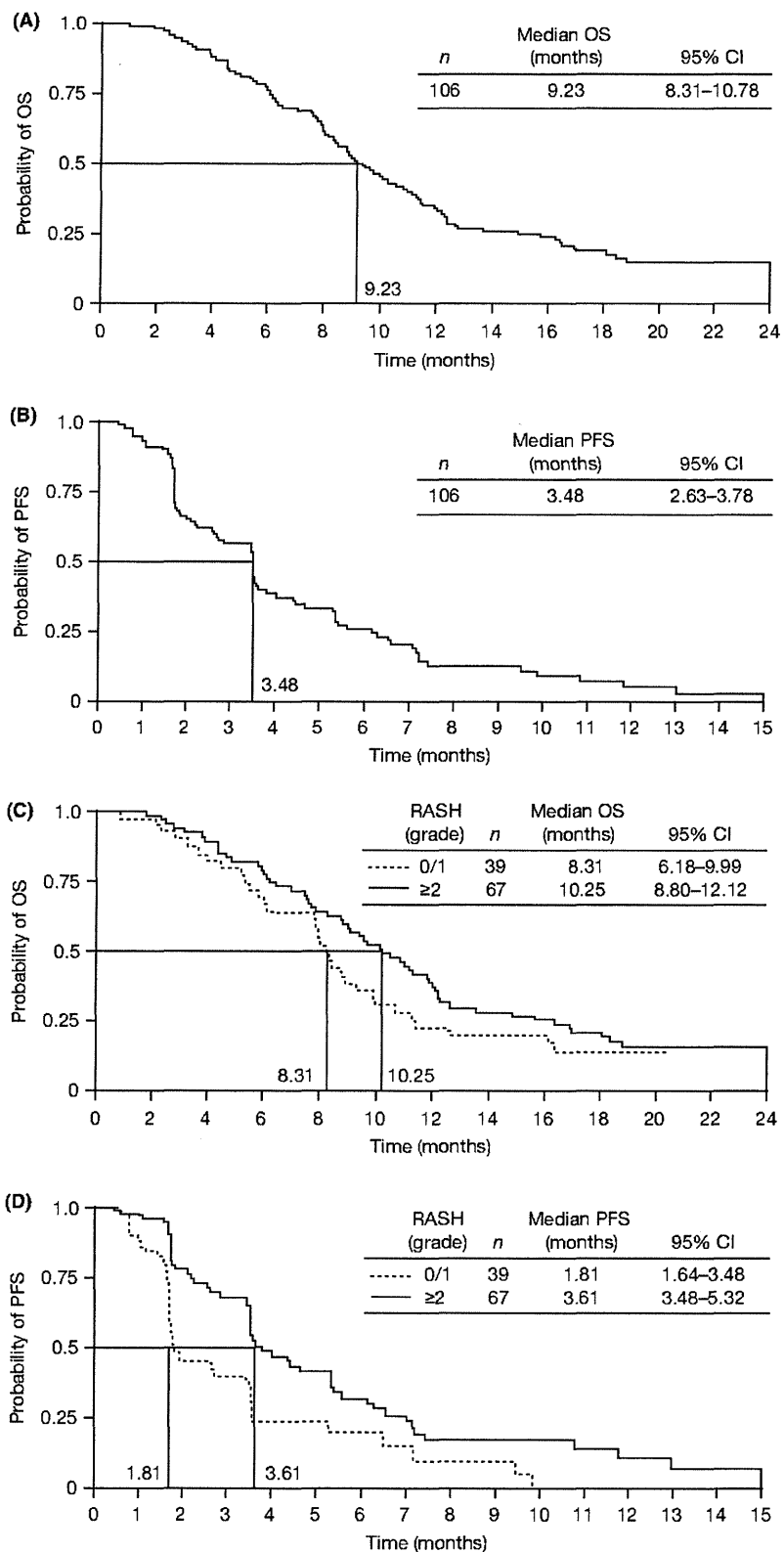
(A)

Probability of OS

n	Median OS (months)	95% CI
106	9.23	8.31–10.78

9.23

Time (months)

(B)

Probability of PFS

n	Median PFS (months)	95% CI
106	3.48	2.63–3.78

3.48

Time (months)

(C)

Probability of OS

RASH (grade)	n	Median OS (months)	95% CI
0/1	39	8.31	6.18–9.99
≥2	67	10.25	8.80–12.12

8.31   10.25

Time (months)

(D)

Probability of PFS

RASH (grade)	n	Median PFS (months)	95% CI
0/1	39	1.81	1.64–3.48
≥2	67	3.61	3.48–5.32

1.81   3.61

Time (months)

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.