

Fig. 2. Detection of *FGFR2* rearrangements. (A) Schematic representation of *FGFR2* gene rearrangements: *FGFR2-AHCYL1* (left) and *FGFR2-BICC1* (right). Arrows indicate the position and direction of the fused genes. Green and red spots indicate the genomic location of 5' and 3' FISH probes for the *FGFR2* gene. (B) Representative FISH pattern of *FGFR2* probes in *FGFR2-AHCYL1* and *FGFR2-BICC1*-positive cases. Arrows indicate a split of 5' green and 3' red signals.

also showed no great distinction between the two groups. However, fusion positive cases had a propensity for hepatitis virus infection (Table 2). Expression of *FGFR2* mRNA was significantly higher in fusion-positive cases than in fusion-negative ones (Supporting Fig. 2). Especially, *KRAS/BRAF* mutant cases showed reduced *FGFR2* expression. This might afford collateral evidence of mutually exclusive relationships between *FGFR2* fusion and *KRAS/BRAF* mutation. Immunohistological analysis revealed prominent *FGFR2* protein expression at both cytoplasm and plasma membrane in fusion-positive cases (Supporting Fig. 3). We further screened 212 gastric cancers, 149 colorectal cancers, and 96 hepatocellular carcinomas by RT-PCR for the presence of these *FGFR2* fusion transcripts. The *FGFR2-BICC1* fusion gene was detected in one colorectal cancer (0.7%) and one hepatocellular carcinoma (1.0%). These fusion-positive non-ICC cases were also hepatitis virus-positive (Table 1).

***FGFR2* Fusions Transform NIH3T3 Cells Both In Vitro and In Vivo.** To assess the oncogenic activity of the *FGFR2* fusion proteins, stable NIH3T3 clones expressing the retrovirally transfected wild-type fusion proteins or their kinase activity-deficient mutants (KD mutant) were established. As shown in Fig. 4A, wild-type *FGFR2-AHCYL1* or *FGFR2-BICC1*-expressing cells showed anchorage-independent colony formation in soft agar, which was severely suppressed in KD mutant expressing cells. Subcutaneous transplantation of these clones into immunodeficient mice resulted

in the formation of tumors from *FGFR2-AHCYL1* and *FGFR2-BICC1* expressing clones, whereas those expressing KD mutants did not form tumors (Fig. 4B).

To investigate the mechanisms by which the *FGFR2* fusion drives oncogenesis, downstream *FGFR* signaling was analyzed *in vitro* (Fig. 5A; Supporting Fig. 4). The wild-type fusion expressing cells showed constitutive tyrosine phosphorylation in the activation loop of the *FGFR* kinase domain. *FGFR2* signaling activates multiple downstream pathways, including RAS/MAPK and PI3K/AKT.¹⁷ Immunoblot analysis revealed that activation of MAPK, but not AKT or STAT3, was induced in clones expressing *FGFR2-AHCYL1* and *FGFR2-BICC1*. These results indicate that *FGFR2* fusion proteins activate

Table 1. Clinical Features of *FGFR2* Fusion Positive Cases

<i>FGFR2</i> fusion	Gender	Age	Virus status	Pathology	Differentiation
<i>FGFR2-AHCYL</i>	F	72	HCV	ICC	mod
<i>FGFR2-AHCYL</i>	F	59		ICC	well
<i>FGFR2-AHCYL</i>	M	62	HCV	ICC	mod
<i>FGFR2-AHCYL</i>	M	73		ICC	well
<i>FGFR2-AHCYL</i>	F	52		ICC	mod
<i>FGFR2-AHCYL</i>	M	59		ICC	well
<i>FGFR2-AHCYL</i>	F	49		ICC	mod
<i>FGFR2-BICC1</i>	M	65	HBV	ICC	mod
<i>FGFR2-BICC1</i>	F	68		ICC	well
<i>FGFR2-BICC1</i>	F	66	HCV	CRC	mod
<i>FGFR2-BICC1</i>	F	46	HBV	HCC	por

ICC: Intrahepatic cholangiocarcinoma

CRC: colorectal cancer

HCC: hepatocellular carcinoma

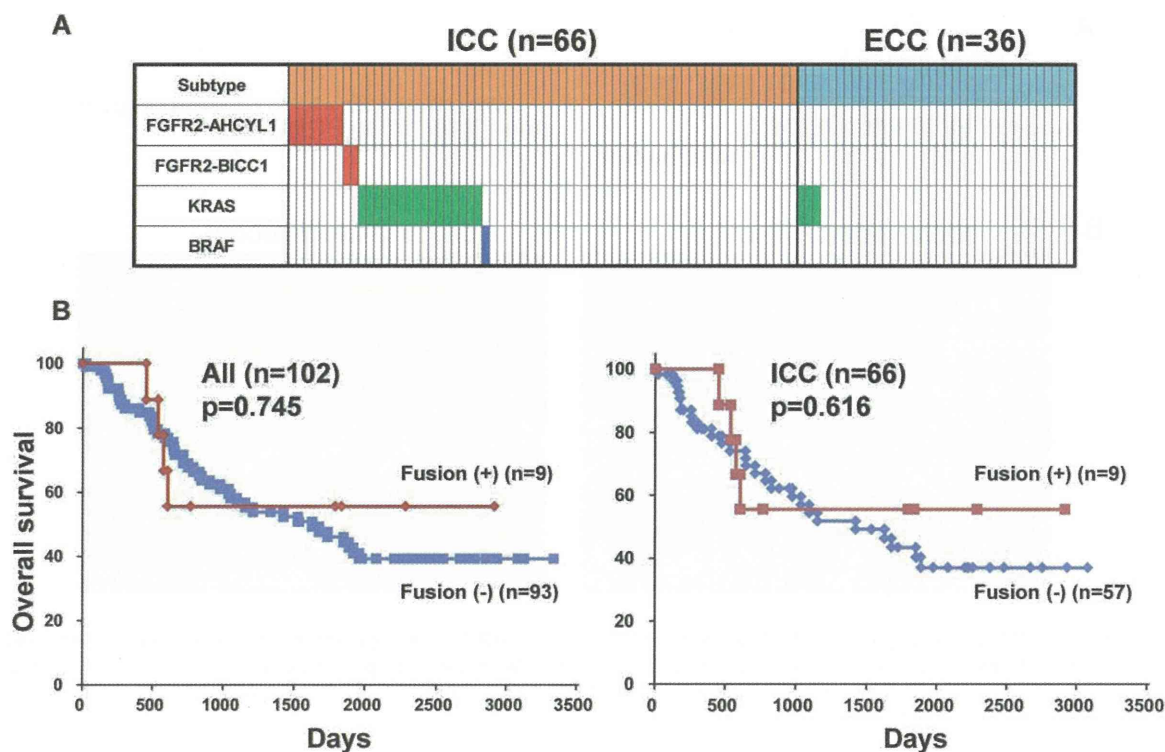


Fig. 3. Clinical subtypes in cholangiocarcinoma. (A) Distribution of genomic alterations. *FGFR2* fusion, *KRAS* mutation, and *BRAF* mutation among ICC and ECC cases are indicated by red, green, and blue, respectively. (B) Overall survival curve stratified by *FGFR2* fusions in all cholangiocarcinoma cases and ICC cases (Kaplan-Meier method). The outcome was not significantly different between *FGFR2* fusion-positive and -negative cases (log-rank test).

canonical FGFR signaling and confer anchorage-independent growth and *in vivo* tumorigenesis, both of which are hallmarks of cellular transformation.

***FGFR2* Fusions Are Potential Therapeutic Targets in Cholangiocarcinoma.** Next, we examined the sensitivity of *FGFR2* fusion-driven tumor cells to two specific FGFR inhibitors, BGJ398 and PD173074, which selectively inhibit FGFR tyrosine kinase activity.^{18,19} These compounds significantly inhibited the phosphorylation of MAPK and reduced *in vitro* anchorage-independent colony formation to the level observed in KD mutant expressing cells (Fig. 5B).

Discussion

FGFR genes are involved in multiple biological processes, ranging from cell transformation, angiogenesis, and tissue repair, to embryonic development. Activating point mutations and amplification of *FGFR* gene members have been explored as therapeutic targets in a wide range of tumors, including bladder, gastric, and lung cancers^{20,21}; however, amplification of *FGFR* genes is uncommon in ICC.²² Diverse fusions involving the *FGFR* gene family have also been reported in hematological and solid cancers^{10,11,23,24} and some have shown sensitivity to FGFR inhibition.

The identification of two recurrent *FGFR2* fusions (*FGFR2-AHCYL1* and *FGFR2-BICC1*) that are mutually exclusive with *KRAS/BRAF* mutations warrants a new molecular classification of cholangiocarcinoma and suggests a novel therapeutic approach in cholangiocarcinomas driven by these fusions. Wu et al.¹¹ recently detected the *FGFR2-BICC1* fusion gene in two cholangiocarcinoma cases, although its prevalence

Table 2. Association Between Clinical Features and *FGFR2* Fusion

Clinical factors		Number of fusion positive case	Number of fusion negative case	P Value
Gender	Male	4	61	0.207
	Female	5	32	
Age (average)		62.1	66.1	0.104
Virus status	Hepatitis virus positive	3	9	0.035
	Hepatitis virus negative	6	84	
Differentiation	Well	4	21	0.367
	Mod	5	60	
	Poor	0	5	
Stage	I	1	2	0.463
	II	2	14	
	III	2	30	
	IV	4	23	

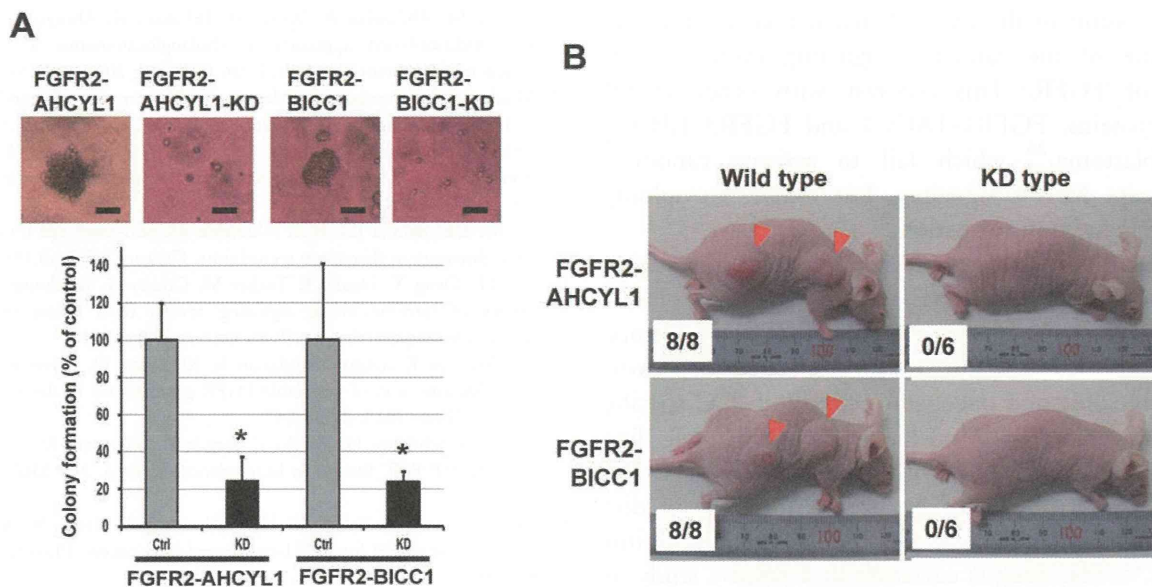


Fig. 4. Oncogenic activity of FGFR2 fusion proteins. (A) Soft agar colony formation in kinase activity-deficient (KD) mutants. The percentage (\pm SD) of colonies with FGFR2 fusions and their KD mutant transfectants are plotted. $*P < 0.05$. A representative image of colonies expressing wild-type and KD FGFR2 fusions is shown (scale bar = 100 μ m). (B) Representative images of mice subcutaneously transplanted with NIH3T3 cells expressing wild-type and KD FGFR2 fusions. The number of tumors per injection in each transfectant is shown.

in cholangiocarcinoma has been lacking. The present study showed a high prevalence of *FGFR2* fusion genes in the intrahepatic subtype of cholangiocarcinoma. Although two cases of another kinase fusion, *FIG-ROS1* (2/23, 8.7%), have been reported by other researchers in CC,¹⁰ we did not detect such fusion in this study. As cholangiocarcinoma is a heterogeneous disease, some epidemiological or clinical specificity may be ascribable to the *FIG-ROS1* fusion. However,

no detailed pathological information of the patients was stated in that study. Further investigation is needed to clarify the whole picture of driver fusion genes in CC. Association between *FGFR2* fusion positivity and hepatitis virus infection may suggest an involvement of the virus in the chromosomal rearrangements in CC. However, rare observation of *FGFR2* fusion in hepatocellular carcinoma argues for further analysis of genetic rearrangements.

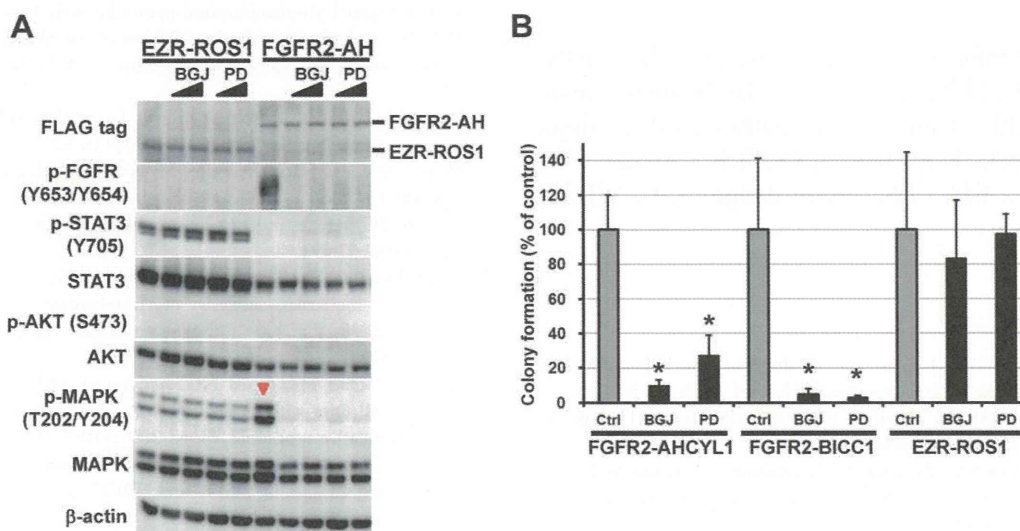


Fig. 5. FGFR inhibitors block signaling in FGFR2-fusion-expressing cells. (A) Activation of FGFR2 and MAPK by FGFR2-AHCYL1 and its suppression by FGFR inhibitors. Lysates from NIH3T3 cells expressing FGFR2-AHCYL1 or EZR-ROS1 (control) treated with vehicle (DMSO), 0.2 and 1 μ M BGJ398, and 0.2 and 1 μ M PD173074 were immunoblotted with the relevant antibodies. β -Actin was used as a loading control. (B) Anchorage-independent growth of NIH3T3 cells expressing FGFR2 fusions and its suppression by FGFR inhibitors (BGJ: BGJ398 and PD: PD173074). The percentage (\pm SD) of colonies formed in the presence of FGFR2 inhibitors (0.2 μ M) with respect to those formed by DMSO-treated cells are plotted. The NIH3T3 clone expressing EZR-ROS1 was used as a negative control for FGFR inhibitors. $*P < 0.05$.

Overexpression of the FGFR2 fusion protein hyperactivate one of the canonical signaling events downstream of FGFR. This contrast with other FGFR fusion proteins, FGFR1-TACC1 and FGFR3-TACC3 in glioblastoma,²⁴ which fail to activate canonical downstream MAPK signaling, but induce aneuploidy and oncogenic transformation.⁹

Based on the specific relevant genomic alterations, TKIs have been developed into effective therapies.^{7,8} We showed that small molecule FGFR inhibitors, BGJ398 and PD173074, efficiently blocked the downstream signaling and oncogenic activity of ICC-specific *FGFR2* fusions. By the high-throughput cell line profiling assay, amplifications or mutations of *FGFR* genes in cancer cell lines have been reported to predict sensitivity to the selective pan-FGFR inhibitor BGJ398.²⁵ This drug is currently in a phase I study in patients of advanced solid tumors with FGFR1/2 amplification or FGFR3 mutation (Novartis, Basel, Switzerland; ClinicalTrials.gov identifier: NCT01004224). Clinical investigations, akin to those conducted in other solid tumors with oncogenic fusion kinases, such as *EML4-ALK*,²⁶ are warranted to examine the efficacy of FGFR inhibitors for the treatment of defined subset of cholangiocarcinoma harboring *FGFR2* fusions.

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References

- Razumilava N, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013;11:13-21.
- Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. *Lancet* 2005;366:1303-1314.
- Ong, CK, Subimerb C, Pairojkul C, Wongkham S, Cutcutache I, et al. Exome sequencing of liver fluke-associated cholangiocarcinoma. *Nat Genet* 2012;44:690-693.
- Voss JS, Holtegaard LM, Kerr SE, Barr Fritcher EG, Roberts LR, Gores GJ, et al. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. *Hum Pathol* 2013;44:1216-1222.
- Hezel AF, Zhu AX. Systemic therapy for biliary tract cancers. *Oncologist* 2008;13:415-423.
- Aljiffry M, Abdulelah A, Walsh M, Peltekian K, Alwayn I, Molinari M. Evidence-based approach to cholangiocarcinoma: a systematic review of the current literature. *J Am Coll Surg* 2009;208:134-147.
- Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer* 2007;7:233-245.
- Abtain J, Nasr R, Bazarbachi A, de The H. The drug-induced degradation of oncoproteins: An unexpected Achilles' heel of cancer cells? *Cancer Discov* 2011;1:117-127.
- Gerber DE, Minna JD. ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. *Cancer Cell* 2010;18:548-551.
- Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rinkunas V, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS One* 2011;6:e15640.
- Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, et al. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov* 2013; 3:636-647.
- Kohno T, Ichikawa H, Totoki Y, Yasuda K, Hiramoto M, Nammo T, et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat Med* 2012;18:375-377.
- Arai Y, Totoki Y, Takahashi H, Nakamura H, Hama N, Kohno T, et al. Mouse model for ROS1-rearranged lung cancer. *PLoS One* 2013; 8:e56010.
- Ando H, Mizutani A, Matsu-ura T, Mikoshiba K. IRBIT, a novel inositol 1,4,5-trisphosphate (IP3) receptor-binding protein, is released from the IP3 receptor upon IP3 binding to the receptor. *J Biol Chem* 2003; 278:10602-10612.
- Wessely O, Tran U, Zakin L, De Robertis EM. Identification and expression of the mammalian homologue of Bicaudal-C. *Mech Dev* 2001;101:267-270.
- Kim CA, Bowie JU. SAM domains: uniform structure, diversity of function. *Trends Biochem Sci* 2003;28:625-628.
- Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin Cancer Res* 2012;18:1855-1862.
- Skaper SD, Kee WJ, Facci L, Macdonald G, Doherty P, Walsh FS. The FGFR1 inhibitor PD 173074 selectively and potently antagonizes FGF-2 neurotrophic and neurotropic effects. *J Neurochem* 2000;75:1520-1527.
- Guagnano V, Furet P, Spanka C, Bordas V, Le Douget M, Stamm C, et al. Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-[6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl]-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. *J Med Chem* 2011;54:7066-7083.
- Turner N, Grose R. Fibroblast growth factor signaling: from development to cancer. *Nat Rev Cancer* 2010;10:116-129.
- Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* 2010;2:62ra93.
- Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, et al. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 2013; 144:829-840.
- Chase A, Grand FH, Cross NC. Activity of TKI258 against primary cells and cell lines with FGFR1 fusion genes associated with the 8p11 myeloproliferative syndrome. *Blood* 2007;110:3729-3734.
- Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science* 2012;337:1231-1235.
- Guagnano V, Kauffmann A, Wöhrle S, Stamm C, Ito M, Barys L, et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. *Cancer Discov* 2012;2:1118-1133.
- Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363:1693-1703.

Randomized Phase III Study of Gemcitabine Plus S-1, S-1 Alone, or Gemcitabine Alone in Patients With Locally Advanced and Metastatic Pancreatic Cancer in Japan and Taiwan: GEST Study

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A B S T R A C T

Purpose

The present phase III study was designed to investigate the noninferiority of S-1 alone and superiority of gemcitabine plus S-1 compared with gemcitabine alone with respect to overall survival.

Patients and Methods

The participants were chemotherapy-naïve patients with locally advanced or metastatic pancreatic cancer. Patients were randomly assigned to receive only gemcitabine (1,000 mg/m² on days 1, 8, and 15 of a 28-day cycle), only S-1 (80, 100, or 120 mg/d according to body-surface area on days 1 through 28 of a 42-day cycle), or gemcitabine plus S-1 (gemcitabine 1,000 mg/m² on days 1 and 8 plus S-1 60, 80, or 100 mg/d according to body-surface area on days 1 through 14 of a 21-day cycle).

Results

In the total of 834 enrolled patients, median overall survival was 8.8 months in the gemcitabine group, 9.7 months in the S-1 group, and 10.1 months in the gemcitabine plus S-1 group. The noninferiority of S-1 to gemcitabine was demonstrated (hazard ratio, 0.96; 97.5% CI, 0.78 to 1.18; $P < .001$ for noninferiority), whereas the superiority of gemcitabine plus S-1 was not (hazard ratio, 0.88; 97.5% CI, 0.71 to 1.08; $P = .15$). All treatments were generally well tolerated, although hematologic and GI toxicities were more severe in the gemcitabine plus S-1 group than in the gemcitabine group.

Conclusion

Monotherapy with S-1 demonstrated noninferiority to gemcitabine in overall survival with good tolerability and presents a convenient oral alternative for locally advanced and metastatic pancreatic cancer.

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INTRODUCTION

Pancreatic cancer (PC) is currently the eighth leading cause of cancer-related mortality worldwide, with an estimated 266,000 deaths in 2008.¹ Gemcitabine became the standard treatment for advanced PC, improving overall survival (OS) compared with fluorouracil.² Although various gemcitabine-based combination regimens have been evaluated, only erlotinib added to gemcitabine showed a survival benefit over gemcitabine, and that was marginal.³

Fluorouracil/leucovorin plus irinotecan plus oxaliplatin (FOLFIRINOX), a gemcitabine-free combination regimen, has recently demonstrated a clear survival benefit compared with gemcitabine for patients with metastatic PC who have a performance status of 0 to 1.⁴ However, because FOLFIRINOX is associated with significant toxicity, this regimen must be limited to patients with good performance status and requires close monitoring.⁵

In Japan, clinical trials of S-1 (TS-1; Taiho Pharmaceutical, Tokyo, Japan) have been conducted since the early 2000s for patients with PC. S-1

GS or S-1 v Gemcitabine for Pancreatic Cancer

is an oral fluoropyrimidine derivative shown to be effective for gastric and various other types of cancers.^{6,7} Phase II studies of S-1 as first-line therapy for metastatic PC resulted in good response rates of 21.1% to 37.5%.^{8,9} Consequently, S-1 was approved for the indication of PC in Japan in 2006. Development of gemcitabine plus S-1 (GS) studies have also been initiated, mainly in Japan, and two phase II studies reported high response rates of 44.4% to 48.5% and good median OS of 10.1 to 12.5 months.^{10,11}

Because S-1 and GS have shown promising activity in PC, the present randomized phase III study (GEST [Gemcitabine and S-1 Trial] study) was designed to evaluate whether S-1 alone is noninferior to gemcitabine and whether GS is superior to gemcitabine alone for locally advanced and metastatic PC with respect to OS.

PATIENTS AND METHODS

Study Design

This randomized phase III study, sponsored by Taiho Pharmaceutical in Japan and TTY Biopharm in Taiwan, was conducted as a postmarketing study in Japan and as a registration study in Taiwan and was in compliance with the Declaration of Helsinki. Data were collected by a contract research organization contracted by the sponsors and were analyzed by a bio-statistician (Y.O.). An independent data and safety monitoring committee reviewed efficacy and safety data. The study was approved by the ethics committee or institutional review board of each participating center.

Patients

All patients provided written informed consent. Enrollment criteria were locally advanced or metastatic PC, histologically or cytologically proven diagnosis of adenocarcinoma or adenosquamous carcinoma, no prior chemotherapy or radiotherapy for PC, age of more than 20 years (the protocol was amended to restrict the eligible age to < 80 years after four of the first eight patients who were \geq 80 years experienced serious adverse events), an Eastern Cooperative Oncology Group performance status score of 0 to 1, and adequate organ functions (see Appendix, online only).

Treatment

Random assignment was performed centrally with stratification by extent of disease (locally advanced disease v metastatic disease) and institution

using the minimization method. Patients allocated to gemcitabine alone received gemcitabine at a dose of 1,000 mg/m² intravenously over 30 minutes on days 1, 8, and 15 of a 28-day cycle. Patients allocated to S-1 alone received S-1 orally twice daily at a dose according to the body-surface area (BSA) (< 1.25 m², 80 mg/d; \geq 1.25 to < 1.5 m², 100 mg/d; \geq 1.5 m², 120 mg/d) on days 1 through 28 of a 42-day cycle. Patients allocated to GS received gemcitabine at a dose of 1,000 mg/m² on days 1 and 8 plus S-1 orally twice daily at a dose according to the BSA (< 1.25 m², 60 mg/d; \geq 1.25 to < 1.5 m², 80 mg/d; \geq 1.5 m², 100 mg/d) on days 1 through 14 of a 21-day cycle. The dose levels of S-1 used in the GS group were based on the results of a previous phase II study of GS, in which 1,000 mg/m² of gemcitabine was combined with 120 mg/d, 100 mg/d, and 80 mg/d of S-1. In that study, the rate of treatment withdrawal due to adverse events was 41% (22 of 54 patients), the rate of grade 3 or worse neutropenia was 80%, and the dose was reduced in 56% of the patients (30 of 54 patients).¹¹ Consequently, 20 mg/d lower doses of S-1 than those used in the S-1 monotherapy group were used in the GS group in the present study.

In the event of predefined toxic events, protocol-specified treatment modifications were permitted (see Appendix).

Assessments

Physical examinations, CBCs, and biochemistry tests were usually checked at 2-week intervals in the S-1 group and at each time of administration of gemcitabine both in the gemcitabine group and in the GS group. All adverse events were assessed according to the Common Terminology Criteria for Adverse Events, version 3.0. Computed tomography or magnetic resonance imaging was performed every 6 weeks until disease progression, and response was assessed by the investigators according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0.¹² Quality of life was assessed using the EuroQol 5 Dimension questionnaire¹³ at baseline and 6, 12, 24, 48, and 72 weeks after the study treatment had begun.

Statistical Analysis

The primary end point was OS, defined as time from date of random assignment to date of death from any cause. Secondary end points were progression-free survival (PFS), objective response rate, safety, and quality of life. PFS was counted from the date of random assignment to the date of death without progression or of progression as confirmed by the investigator's assessment. The median OS was assumed to be 7.5 months in the gemcitabine group, 8.0 months in the S-1 group, and 10.5 months in the GS group. To maintain a one-sided significance level of .025 for the entire study while testing two hypotheses (ie, noninferiority and superiority), the one-sided significance

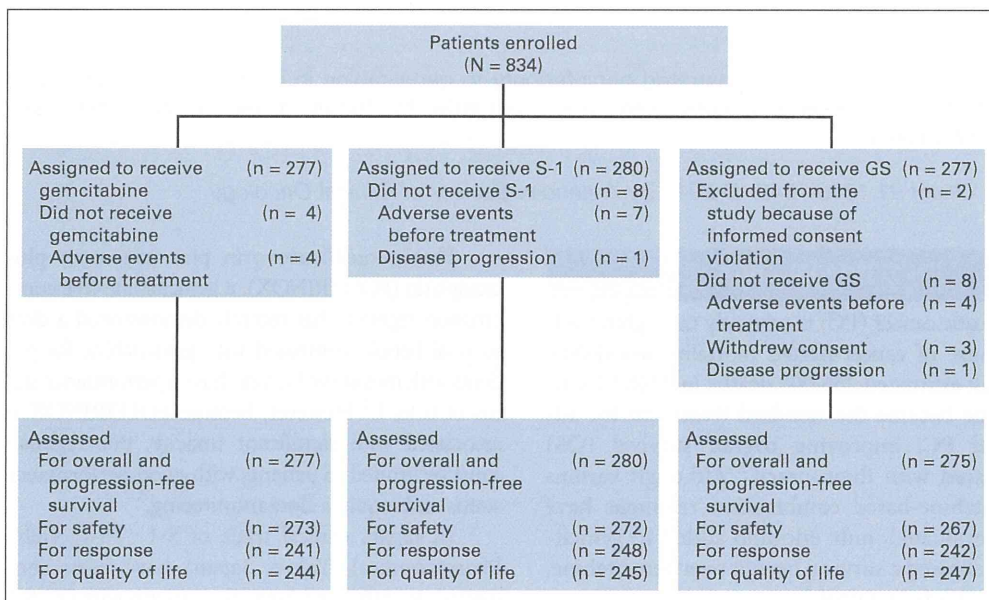


Fig 1. CONSORT diagram. GS, gemcitabine plus S-1.

level for each comparison was set at .0125. The statistical considerations are detailed in the Appendix.

The superiority of GS was evaluated by the stratified log-rank test. To assess the noninferiority of S-1, we used the Cox proportional hazards model to calculate two-sided, 97.5% CIs of the hazard ratio (HR). The noninferiority margin of S-1 was set at 1.33; that is, the null hypothesis was that the median OS with S-1 would be approximately 2 months shorter than with gemcitabine. We decided this setting was justified considering the convenience of S-1 and because there are few effective drugs for the disease. Furthermore, to interpret the obtained data, the Bayesian analysis of the log HR on the basis of the noninformative prior distribution was preplanned. Posterior probability with log HR within a stricter threshold (log 1.15) was also calculated.¹⁴

In each assigned group, the time-to-event distribution was estimated with the Kaplan-Meier method. The 95% CI of the median survival time was calculated by the method of Brookmeyer and Crowley.¹⁵ In addition, the Greenwood formula¹⁶ was used to calculate the 95% CI for survival rates. In subgroup analyses, interaction tests were performed to assess the homogeneity of the effect of treatment on OS.

The primary end point was analyzed for the full analysis set. All *P* value evaluations were two-tailed. Data analyses were done with SAS, version 9.1.3 (SAS Institute, Cary, NC).

RESULTS

Patients

Between July 2007 and October 2009, a total of 834 patients were enrolled from 75 institutions in Japan and Taiwan (768 in Japan and 66 in Taiwan). Two patients in the GS group were excluded from the study because enrollment was conducted before obtaining written informed consent. The remaining 832 patients were included in the full analysis set and used to calculate OS and PFS (Fig 1). The three treatment groups were well balanced with respect to demographic and baseline characteristics (Table 1).

Study Treatment

The median duration of treatment was 2.6 months in the gemcitabine group, 2.6 months in the S-1 group, and 4.3 months in the GS group. The main reasons for treatment discontinuation were either disease progression (202 patients [72.9%] in the gemcitabine group,

Table 1. Demographics and Baseline Characteristics of Patients (full-analysis set population)

Characteristic	Gemcitabine (n = 277)		S-1 (n = 280)		GS (n = 275)		Total (N = 832)	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	170	61.4	170	60.7	158	57.5	498	59.9
Female	107	38.6	110	39.3	117	42.5	334	40.1
Age, years								
< 65	134	48.4	145	51.8	137	49.8	416	50.0
≥ 65	143	51.6	135	48.2	138	50.2	416	50.0
ECOG PS								
0	181	65.3	178	63.6	172	62.5	531	63.8
1	96	34.7	102	36.4	103	37.5	301	36.2
Extent of disease								
Locally advanced	66	23.8	68	24.3	68	24.7	202	24.3
Metastatic	211	76.2	212	75.7	207	75.3	630	75.7
Type of tumor								
Adenocarcinoma	272	98.2	276	98.6	272	98.9	820	98.6
Adenosquamous carcinoma	5	1.8	4	1.4	3	1.1	12	1.4
Pancreas excision								
No	254	91.7	264	94.3	248	90.2	766	92.1
Yes	23	8.3	16	5.7	27	9.8	66	7.9
Tumor location*								
Head	122	44.0	110	39.3	116	42.2	348	41.8
Body	88	31.8	124	44.3	102	37.1	314	37.7
Tail	68	24.5	55	19.6	66	24.0	189	22.7
Biliary drainage								
No	202	72.9	217	77.5	209	76.0	628	75.5
Yes	75	27.1	63	22.5	66	24.0	204	24.5
CEA, ng/mL								
Median	5.7		5.6		5.9		5.7	
IQR	3.0-20.1		2.5-18.4		2.5-20.7		2.6-19.5	
CA19-9, U/mL								
Median	1,044		726		441		712	
IQR	52-5,002		64-5,000		45-5,090		55-5,002	
CRP, mg/dL								
Median	0.40		0.50		0.40		0.43	
IQR	0.11-1.38		0.18-1.57		0.15-1.60		0.15-1.57	

Abbreviations: CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRP, C-reactive protein; ECOG PS, Eastern Cooperative Oncology Group performance status; GS, gemcitabine plus S-1; IQR, interquartile range.

*Including patients with tumors involving multiple sites.

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215 [76.8%] in the S-1 group, and 162 [58.9%] in the GS group) or adverse events (40 patients [14.4%] in the gemcitabine group, 38 [13.6%] in the S-1 group, and 76 [27.6%] in the GS group). The median relative dose-intensity was 83.0% in the gemcitabine group, 96.1% in the S-1 group, and 83.3% for gemcitabine and 87.4% for S-1 in the GS group.

Survival

The median duration of follow-up for surviving patients was 18.4 months (range, 0.3 to 36.9 months) as of July 31, 2010. The analysis of OS was based on 710 deaths (85.3%) among the 832 patients. The median OS was 8.8 months (95% CI, 8.0 to 9.7) in the gemcitabine group, 9.7 months (95% CI, 7.6 to 10.8) in the S-1 group, and 10.1 months (95% CI, 9.0 to 11.2) in the GS group (Fig 2A). OS rates at 12 and 24 months were respectively 35.4% and 9.2% in the gemcitabine group, 38.7% and 12.7% in the S-1 group, and 40.7% and 14.5% in the GS group. The noninferiority of S-1 to gemcitabine with respect to OS was demonstrated (HR, 0.96; 97.5% CI, 0.78 to 1.18; $P < .001$ for

noninferiority). The Bayesian posterior probability that the HR of S-1 relative to gemcitabine would be less than 1.15 was calculated to be 98% on the basis of the noninformative prior distribution. However, GS failed to improve OS at a statistically significant level as compared with gemcitabine (HR, 0.88; 97.5% CI, 0.71 to 1.08; $P = .15$).

The analysis of PFS was based on 793 events (95.3%) among the 832 patients. The median PFS was 4.1 months (95% CI, 3.0 to 4.4) in the gemcitabine group, 3.8 months (95% CI, 2.9 to 4.2) in the S-1 group, and 5.7 months (95% CI, 5.4 to 6.7) in the GS group (Fig 2B). PFS rates at 6 and 12 months were respectively 29.8% and 9.1% in the gemcitabine group, 26.9% and 7.2% in the S-1 group, and 47.9% and 20.3% in the GS group. S-1 was shown to be noninferior to gemcitabine with respect to PFS (HR, 1.09; 97.5% CI, 0.90 to 1.33; $P = .02$ for noninferiority), and GS significantly improved PFS compared with gemcitabine (HR, 0.66; 97.5% CI, 0.54 to 0.81; $P < .001$).

Subgroup analyses of survival according to pretreatment characteristics showed no significant interaction between S-1 and gemcitabine in any subgroup (Fig 3A). However, GS showed a favorable HR compared with gemcitabine in the subsets of patients with locally advanced disease or patients with a performance status of 1 (Fig 3B).

Response to Therapy

The objective response rate was 13.3% (95% CI, 9.3 to 18.2) in the gemcitabine group, 21.0% (95% CI, 16.1 to 26.6) in the S-1 group, and 29.3% (95% CI, 23.7 to 35.5) in the GS group (Table 2). The objective response rate was significantly higher in the S-1 group ($P = .02$) and in the GS group ($P < .001$) than in the gemcitabine group.

Second-Line Chemotherapy

Second-line chemotherapy was performed in 184 patients (66.4%) in the gemcitabine group, 185 (66.1%) in the S-1 group, and 172 (62.5%) in the GS group. In the gemcitabine group, 140 patients (50.5%) received S-1 alone or S-1–based regimens, and in the S-1 group 162 (57.9%) received gemcitabine alone or gemcitabine–based regimens as second-line chemotherapy. The most common second-line regimens in the GS group were gemcitabine alone (61 patients), GS (53 patients), S-1 alone (24 patients), irinotecan (six patients), and fluorouracil/leucovorin plus oxaliplatin (four patients). In Japan and Taiwan, the use of treatments such as erlotinib, oxaliplatin, and irinotecan for PC was not approved at the time of this study; hence gemcitabine, S-1, or both were used in most patients as second-line chemotherapy.

Adverse Events and Quality-Adjusted Life-Years

The major grade 3 or worse adverse events are listed in Table 3. Patients in the gemcitabine group had significantly higher incidences of grade 3 or worse leukopenia, neutropenia, thrombocytopenia, elevated AST levels, and elevated ALT levels as compared with patients in the S-1 group. However, the incidence of grade 3 or worse diarrhea was higher in the S-1 group than in the gemcitabine group. Patients in the GS group had significantly higher incidences of grade 3 or worse leukopenia, neutropenia, thrombocytopenia, rash, diarrhea, vomiting, and stomatitis than patients in the gemcitabine group.

There were three deaths considered possibly related to the protocol treatment (interstitial lung disease, sepsis, and acute hepatitis B) in the gemcitabine group, one in the S-1 group (unknown cause), and

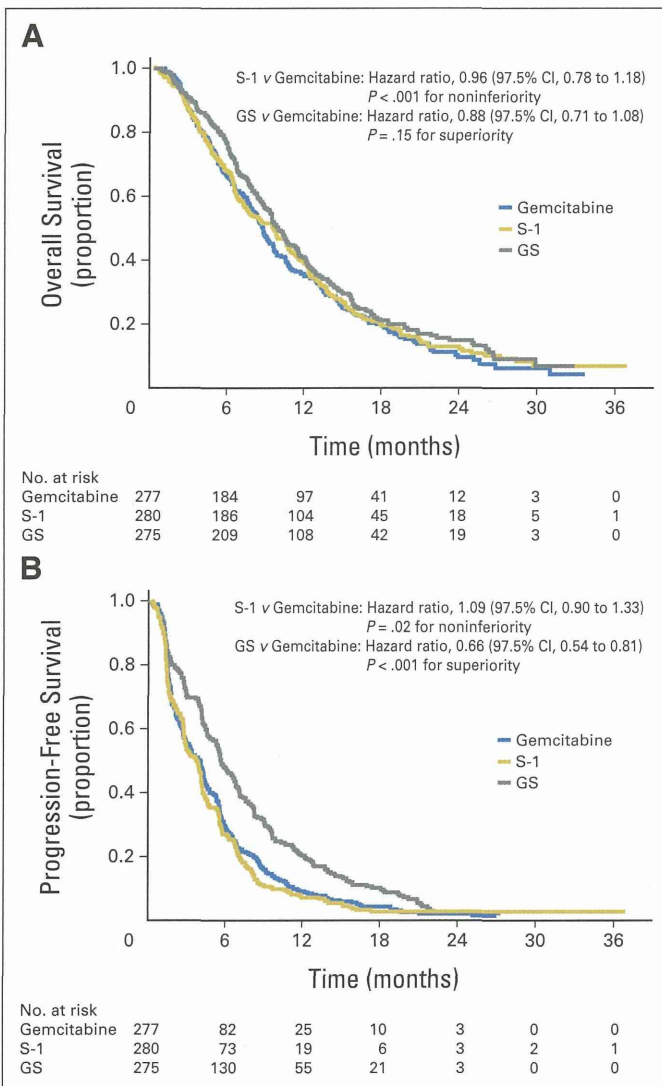


Fig 2. Kaplan-Meier estimates of (A) overall survival and (B) progression-free survival according to treatment group. GS, gemcitabine plus S-1.

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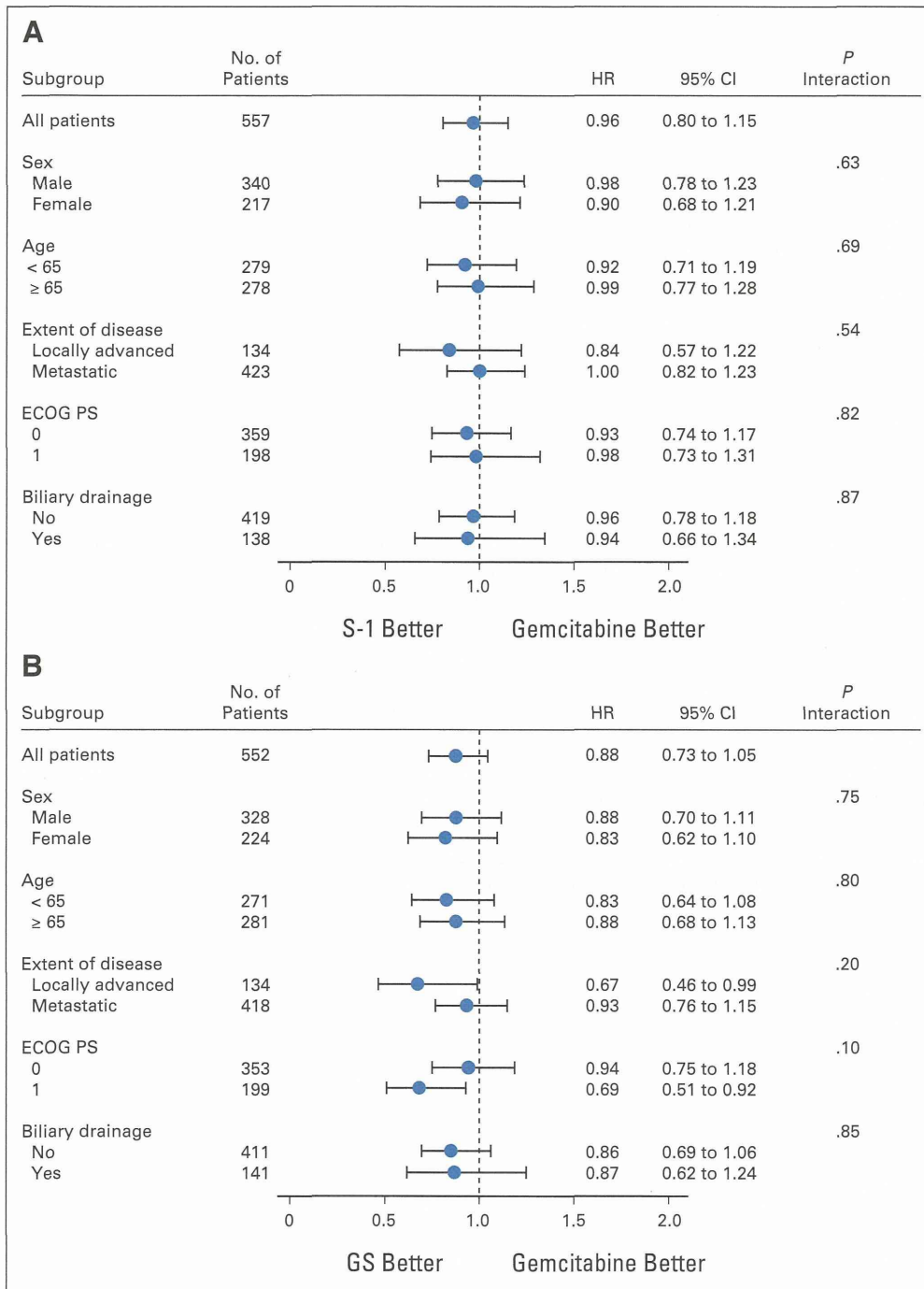


Fig 3. Forest plots of treatment effects on overall survival in subgroup analyses. Forest plots show effects on overall survival of patients in each subgroup. (A) S-1; (B) gemcitabine plus S-1 (GS). Each blue circle shows the treatment response. ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio.

four in the GS group (unknown cause associated with myelosuppression, cerebral infarction, cerebrovascular disorder, and interstitial lung disease). The results of quality-adjusted life-years (QALYs) are in the Appendix and the details of quality-of-life assessments will be reported elsewhere.

DISCUSSION

The overall and PFS curves in the S-1 group were nearly identical to those in the gemcitabine group, confirming the noninferiority of S-1

to gemcitabine in terms of OS and PFS (Fig 2A, 2B). Toxicity profiles of these two drugs differed slightly: gemcitabine tended to show hematologic toxicity, whereas S-1 tended to show GI toxicity. However, both S-1 and gemcitabine were generally well tolerated. Furthermore, the results of QALY evaluation demonstrated that S-1 and gemcitabine were equivalent. Hence our results suggest that S-1 can be used as first-line therapy as a convenient oral alternative for locally advanced and metastatic PC. To the best of our knowledge, this is the first phase III study to demonstrate the noninferiority of a single anticancer agent to gemcitabine alone for locally advanced and metastatic PC.

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Table 2. Objective Response Rates (patients with measurable lesions)

Variable	Gemcitabine (n = 241)		S-1 (n = 248)		GS (n = 242)		<i>P</i> (χ^2 test)	
	No.	%	No.	%	No.	%	Gemcitabine v S-1	Gemcitabine v GS
Response								
Complete response	1	0.4	0	0	2	0.8		
Partial response	31	12.9	52	21.0	69	28.5		
Stable disease	119	49.4	105	42.3	102	42.1		
Progressive disease	75	31.1	69	27.8	37	15.3		
Objective response rate*	32	13.3	52	21.0	71	29.3	.02	< .001
95% CI	9.3 to 18.2		16.1 to 26.6		23.7 to 35.5			
Disease control rate†	151	62.7	157	63.3	173	71.5	.88	.04
95% CI	56.2 to 68.8		57.0 to 69.3		65.4 to 77.1			

Abbreviation: GS, gemcitabine plus S-1.

*The objective response rate was defined as the proportion of patients who had a complete response or partial response.

†The disease control rate was defined as the proportion of patients who had a complete response, partial response, or stable disease.

At the time of planning this study, the participants of nearly all phase III trials included both patients with locally advanced as well as those with metastatic PC. However, because locally advanced and metastatic diseases are two clinical entities, it is recently recommended that patients with locally advanced disease should be studied separately from those with metastatic disease.¹⁷ Although this study included locally advanced disease, subgroup analysis of extent of disease showed no significant interaction between S-1 and gemcitabine (Fig 3A). Moreover, the OS curve in the S-1 group was still similar to those in the gemcitabine group in both locally advanced and metastatic disease (Fig 4A, 4B). Regarding pathologic diagnosis, our study included adenosquamous carcinoma, although its percentage was very low (1.4% of whole population). When the data were reanalyzed after

excluding patients with adenosquamous carcinoma, the results for OS for gemcitabine versus S-1 was unchanged (HR, 0.96; 95% CI, 0.81 to 1.15). The selection of one treatment over the other will depend primarily on patient preference, clinical factors, or drug costs, as biomarkers indicating effective use of S-1 or gemcitabine do not exist at this time.

Regarding GS, the OS did not differ significantly from gemcitabine, although the PFS was significantly longer in the GS group. Second-line chemotherapy mainly with S-1 in the gemcitabine group may be one reason for this discrepancy. The median OS in the gemcitabine group was 8.8 months, which is longer than those previously reported for gemcitabine in other phase III studies for locally advanced and metastatic PC.^{2,3,18-24} Although the efficacy of second-line

Table 3. Grade 3 or Worse Adverse Events (safety population)

Event	Gemcitabine (n = 273)		S-1 (n = 272)		GS (n = 267)		<i>P</i> (Fisher's exact test)	
	No.	%	No.	%	No.	%	Gemcitabine v S-1	Gemcitabine v GS
Hematologic								
Leukocytes	51	18.7	10	3.7	101	37.8	< .001	< .001
Neutrophils	112	41.0	24	8.8	166	62.2	< .001	< .001
Platelets	30	11.0	4	1.5	46	17.2	< .001	.05
Hemoglobin	39	14.3	26	9.6	46	17.2	.11	.41
Nonhematologic								
ALT	41	15.0	16	5.9	29	10.9	< .001	.16
AST	41	15.0	21	7.7	32	12.0	.01	.32
Bilirubin	26	9.5	39	14.3	23	8.6	.09	.77
Fatigue	10	3.7	18	6.6	13	4.9	.13	.53
Rash	2	0.7	2	0.7	11	4.1	1.00	.01
Anorexia	20	7.3	31	11.4	25	9.4	.11	.44
Diarrhea	3	1.1	15	5.5	12	4.5	.004	.02
Mucositis/stomatitis	0	0.0	2	0.7	6	2.2	.25	.01
Nausea	5	1.8	5	1.8	12	4.5	1.00	.09
Vomiting	2	0.7	4	1.5	12	4.5	.45	.006
Febrile neutropenia	1	0.4	1	0.4	5	1.9	1.00	.12
Infection with normal ANC	6	2.2	7	2.6	6	2.2	.79	1.00
Pneumonitis	5	1.8	0	0.0	2	0.7	.06	.45

NOTE. Grades of adverse events were defined according to the Common Terminology Criteria for Adverse Events (version 3.0).

Abbreviations: ANC, absolute neutrophil count; GS, gemcitabine plus S-1.