dressed[12]. Our results confirmed the usefulness of EUS-FNA, especially with regard to cytology. The National Comprehensive Cancer Network Guidelines (2012) require that cytological or histological confirmation is needed for the diagnosis of unresectable pancreatic carcinoma[13]. In patients with stage IV PC, a biopsy of the metastatic lesion is preferred for proof of cancer. However, in those with stage III PC and some patients with stage IV PC in whom it is difficult to access metastatic sites for biopsy procedures, the primary tumor of the pancreas must be targeted to obtain proof of cancer. Pancreatic juice cytology was developed in the early 1980s and is still being performed; however, cancer cells cannot easily be observed by collection of pancreatic juice[1,2,14]. Percutaneous needle biopsy was developed with the expectation of a more definitive method to obtain proof of cancer from the primary pancreatic tumor[3,15,16]. Our institute then used percutaneous needle biopsy under extracorporeal US guidance as the standard for histological confirmation of the pancreatic primary tumor. Recently, EUS-FNA was introduced and was used mainly in high-volume cancer centers in Japan[17-22]. As a result of the risk of cancer seeding as well as other risks with percutaneous biopsy, we adopted EUS-FNA beginning in November 2009 in place of percutaneous biopsy. We expected that EUS-FNA would have advantages over a percutaneous procedure with regard to efficacy in confirmation of cancer and avoiding adverse reactions before administering chemotherapy to patients with PC.

Our results demonstrated that EUS-FNA is effective and feasible for obtaining proof of cancer in candidates for PC chemotherapy. In fact, EUS-FNA might have merits with regard to obtaining specimens from small tumors or tumors in the pancreatic tail, for which performance of percutaneous biopsy is difficult[2,23-27]. In this study, the location of the target tumor was most frequent at the body of the pancreas in Group A. In addition, the target tumors were larger in Group A than in Group B. These findings suggest that patients might have been excluded from Group A in which difficulty could be expected in making a puncture because the tumor was either small or difficult to delineate. In these cases, endoscopic retrograde cholangiopancreatography or liver biopsy might have been performed to obtain confirmation of malignancy, if possible.

Horwhat *et al*[12] have performed a randomized controlled trial of EUS-FNA and percutaneous biopsy of the pancreas (US- and CT-guided) in 2006. Although there was no statistically significant difference in accuracy between the two methods, the results showed that EUS-FNA had the advantage in the diagnosis of pancreatic malignancy. In our study, the diameters of the target tumors in the EUS-FNA group (Group B) were smaller than those in the US-FNA group (Group A) and the deviation of distribution around the puncture site was smaller in the EUS-FNA than the US-FNA group. Our results indicated high performance through the use of EUS-FNA and are not inconsistent with those of Horwhat *et al*[12]. In the present study, there was no analysis of accuracy in the two groups, because our institution is an oncology hospital and we rarely perform biopsies of benign cases.

The benefits of EUS-FNA might be maximized to make a pathological diagnosis in patients with an abdominal tumor of an uncertain type. The definite merit of our EUS-FNA procedure was thought to be rapid cytological results, but perhaps success in this regard was mainly due to the contribution of an on-site cytotechnologist and not to the EUS-FNA procedure itself. Iglesias-Garcia *et al*[28] have claimed that on-site cytological evaluation improves the diagnostic yield of EUS-guided FNA for the cytological diagnosis of solid pancreatic masses. Savoy *et al*[29] have pointed out that even trained endosonographers have variable and, in some cases, inferior abilities in interpreting on-site cytology in comparison with cytotechnologists. In the present study, we had adequate specimens for all cases in the EUS-FNA group. This is natural because we continued the examination until we obtained a sufficient quantity of specimens that were checked by the on-site cytotechnologist. On the contrary, there was no difference in the rate of adequate specimens obtained for histological examination between the EUS-FNA and US-FNA groups, because the collected tissue was checked by the examiner's naked eye in both groups. The presence of an on-site cytotechnologist to accompany EUS-FNA is considered to be necessary, at least, in high-volume centers.

In the present study, the positivity rate for malignancy was higher for EUS-FNA cytology than for histology. Supporting the current results, another study has shown that the positivity rate for malignancy in EUS-FNA cytology of the pancreas was higher than that in histology[30].

As previously reported, EUS-needle core biopsy is useful for histological and cytological diagnosis in terms of sample volume[31]. In addition, the combined results of EUS-FNA cytology and EUS-needle core biopsy have been reported to improve diagnosis[32-34]. However, to confirm the malignancy, EUS-FNA cytology is more useful than EUS-needle core biopsy[35]. This result is similar to the results of our study, indicating that cytology might be more useful than histology for the diagnosis of malignancy.

In the current study, there was no cancer seeding in any patient in either group. As previously reported, there were rare cases of seeding among patients who underwent US-guided FNA[36]. With regard to the puncture route, we suggest that there is less possibility of seeding in patients who undergo EUS-FNA than in patients who undergo US-FNA, although some recent studies have shown the possibility of seeding in patients who undergo EUS-FNA[37-39]. We did inform patients who were scheduled to undergo EUS-FNA about the possibility of this complication.

The limitations of our study included its retrospective nature. Furthermore, there were no cases of benign pancreatic conditions to enable an evaluation of US and EUS-FNA for accurate differentiation between malignant

and benign diseases.

In conclusion, EUS-FNA, as well as percutaneous needle aspiration, is an effective modality to obtain cytopathological confirmation in patients with advanced PC. EUS-FNA cytology was able to detect malignancy at a high rate. We believe that EUS-FNA has advantages for smaller tumors located deeply and for tumors in which the diagnosis is uncertain by various other imaging modalities.

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COMMENTS

Background

Ultrasonography-guided fine-needle aspiration (US-FNA) biopsy or computed tomography (CT)-guided FNA biopsy was used for histological/cytological diagnosis of pancreatic cancer (PC). US-FNA is limited to masses in the pancreatic tail. CT-guided FNA is time-consuming and limited by a substantial false-negative rate. There have been concerns about percutaneous cancer seeding and difficulty in puncturing for small tumors. Endoscopic ultrasound (EUS)-guided FNA has been developed as a more feasible method of obtaining definitive specimens for the diagnosis of PC. Studies on the results of the two different approaches to obtain pancreatic biopsy specimens, which are the percutaneous approach and EUS-FNA, have rarely been conducted.

Research frontiers

The benefits of EUS-FNA might be maximized to be able to make a pathological diagnosis in patients with an abdominal tumor of an uncertain type.

Innovations and breakthroughs

EUS-FNA is effective and feasible for obtaining proof of cancer in PC chemotherapy candidates. In fact, EUS-FNA might have advantages with regard to obtaining specimens from small tumors or tumors in the pancreatic tail, for which performance of percutaneous biopsy is difficult.

Applications

The results suggest that EUS-FNA is the best method of obtaining cytological samples for diagnosis of unresectable PC. This method can be used for other types of cancer.

Terminology

On-site cytotechnologist: An on-site cytotechnologist should attend the puncture examination to confirm quickly the existence of atypical cells. The information of the cytotechnologist is more appropriate than that of the endoscopist.

Peer review

This is a good descriptive study in which EUS-FNA is a feasible and safe technique to acquire pancreatic specimens. The results are interesting in that the advantages of EUS-FNA over the percutaneous procedure are time between examination and diagnosis, the possibility of puncture of small tumors, and tumors in the tail of the pancreas.

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PHASE II STUDIES

Phase II study of sunitinib in Japanese patients with unresectable or metastatic, well-differentiated pancreatic neuroendocrine tumor

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Summary *Background.* Pancreatic neuroendocrine tumors (NETs) are rare but are frequently diagnosed at advanced stages and require systemic therapy. *Patients and methods.* This multicenter, open-label, phase II study evaluated sunitinib in Japanese patients with well-differentiated pancreatic NET. Patients received sunitinib 37.5 mg/day on a continuous daily dosing (CDD) schedule. The primary endpoint was clinical benefit rate (CBR; percentage of complete responses [CRs] plus partial responses [PRs] plus stable disease [SD] ≥24 weeks). Secondary endpoints included objective response rate (ORR), tumor shrinkage, progression-free survival (PFS) probability, safety, pharmacokinetics, and biomarkers. *Results.* Twelve patients received treatment. The CBR was 75 % (95 % confidence interval [CI], 43–94) and included 6 patients with a PR and 3 with SD. The ORR was 50 % (95 % CI, 21–79). PFS probability was 91 % (95 % CI, 54–99) at 6 months and 71 % (95 % CI, 34–90) at 12 months. Commonly reported treatment-emergent (all-causality), any-grade adverse events included diarrhea ($n=10$), hand–foot syndrome and hypertension (both $n=8$), fatigue and headache (both $n=7$), and neutropenia ($n=6$). No deaths on study were reported; one death due to disease progression occurred >28 days after end of treatment. Sunitinib on a CDD schedule resulted in sustained drug concentrations without accumulation across cycles. Tumor

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responses in all 12 patients did not appear to correlate with decreases in chromogranin A levels. *Conclusions.* Sunitinib 37.5 mg/day on a CDD schedule demonstrated antitumor activity in Japanese patients with unresectable, well-differentiated pancreatic NET. Commonly reported adverse events were consistent with the known safety profile of sunitinib.

Keywords Efficacy · Japanese · Pancreatic neuroendocrine tumor · Pharmacokinetics · Phase II · Sunitinib

Introduction

Pancreatic neuroendocrine tumors (NETs) are rare malignancies with a prevalence of 2.23 per 100,000 population in Japan [1]. The incidence rate of pancreatic NET per year in Japan (1.01 per 100,000 population) appears higher than in Western countries (0.32/100,000 in the overall US population and 0.25 in Asian Americans [2]). Surgery, if feasible, is the optimal treatment approach [3]. However, the majority of patients present with unresectable disease. When the current study was initiated, treatment options available for symptomatic patients with unresectable disease included somatostatin analogs (e.g. octreotide, alone or in combination with interferon-alpha) and the alkylating agent streptozocin (alone or in combination with doxorubicin), both of which have limited efficacy in patients with advanced disease [4–6]. Subsequently, targeted anticancer agents have been shown to improve progression-free survival (PFS) compared with placebo in phase III studies that included primarily Caucasian patients with advanced pancreatic NET [7–9].

Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are key drivers of angiogenesis in pancreatic NETs [10, 11]. Sunitinib malate (SUTENT®), an oral multitargeted inhibitor of numerous receptor tyrosine kinases including VEGF receptors and PDGF receptors [12–14], has been shown to delay tumor growth in a RIP1-Tag2 transgenic mouse model of pancreatic islet-cell tumors [15, 16]. In a phase II trial, sunitinib demonstrated antitumor activity in patients with pancreatic NET [17], and in a subsequent phase III trial, oral sunitinib 37.5 mg/day on a continuous daily dosing (CDD) schedule prolonged median PFS relative to placebo in Caucasian and Asian patients with locally advanced and/or metastatic, well-differentiated pancreatic NET [7]. Sunitinib was also associated with a greater objective tumor response rate than placebo. In an updated analysis, median overall survival (OS) favored sunitinib (hazard ratio [HR] 0.71, 95 % confidence interval [CI]: 0.47–1.09; $P=0.11$), despite crossover to sunitinib for most of the patients randomized to placebo, although statistical significance was not reached [18]. On the basis of these findings, sunitinib has been approved multinationally for the treatment of patients with unresectable or metastatic, well-differentiated pancreatic NET with disease progression.

We carried out a phase II, open-label, multicenter trial (NCT01121562) to evaluate the clinical benefit rate (CBR) of sunitinib in Japanese patients with pancreatic NET. The sunitinib dose investigated was 37.5 mg/day on the CDD schedule, which was the same regimen used in a Western phase III study [7]. Secondary objectives were to assess objective response rate (ORR) and PFS, to evaluate safety and tolerability, and to determine the pharmacokinetic (PK) profile of sunitinib in this patient population.

Patients and methods

Study population

Japanese patients ≥20 years old with histologically or cytologically proven, well-differentiated pancreatic NET (according to the World Health Organization 2004 classification [19]) and progressive unresectable advanced or metastatic disease were eligible to participate. Inclusion criteria comprised documented evidence of disease progression within 12 months of study start (by computed tomography [CT] or magnetic resonance imaging [MRI]), and disease not amenable to surgery, radiation, or combined modality therapy with curative intent. At least one measurable target lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 [20] was required, along with adequate organ function, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, and a life expectancy of at least 3 months. Patients were excluded if they had poorly differentiated tumors, prior treatment with any tyrosine kinase or anti-VEGF angiogenic inhibitors, brain metastases, cardiovascular disease ≤12 months prior to study start, uncontrolled hypertension, an uncontrolled thyroid abnormality, ongoing cardiac dysrhythmias with medical intervention or a prolonged QT interval corrected for heart rate (QTc), symptomatic brain metastases, or a left ventricular ejection fraction of ≤50 %.

Study design and treatment

In this multicenter, open-label, phase II study, all patients received oral sunitinib37.5 mg/day on a CDD schedule, and each treatment cycle lasted 28 days. Patients were monitored for toxicity, and dose reductions to 25 mg/day were permitted based on individual tolerability. The sunitinib dose could also be increased to 50 mg/day (if no response was observed in the first 8 weeks and if individual tolerability permitted). The primary endpoint was CBR, defined as the proportion of patients with a confirmed complete response (CR) or partial response (PR) or stable disease (SD) for ≥24 weeks. CBR was selected as the primary endpoint because maintaining

prolonged SD over about half of a year (24 weeks) was deemed beneficial and clinically meaningful for patients with pancreatic NET, based on the median PFS of 5.5 months reported for placebo treatment in a previous global, pivotal, phase III study [7]. Secondary efficacy endpoints included ORR, defined as the proportion of patients with a confirmed CR or PR; tumor shrinkage, defined as the percentage change from baseline in the sum of the longest diameter of target lesions; PFS; safety; and PK.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and applicable local regulatory requirements and laws. Approval from the institutional review board or independent ethics committee of each participating center was required, and written informed consent was obtained from all patients before screening.

Study assessments

Investigator-assessed tumor imaging by CT, spiral CT, or MRI was performed at screening and weeks 5 and 9, and then at 8-week intervals during the study. Additional scans were performed when disease progression was suspected or to confirm a CR or PR based on RECIST. Safety was assessed at regular intervals by physical examination and analysis of adverse events (AEs), laboratory abnormalities (hematology and blood chemistry), vital signs, 12-lead electrocardiograms (ECGs), and ECOG PS. AEs were graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. QTc intervals were determined using 12-lead ECGs in triplicate at baseline, on day 1 of cycles 2 and 3, every 8 weeks thereafter, and as clinically indicated.

Blood samples were collected before dosing on day 15 (±1) of cycle 1 and on day 1 of cycles 2–4 to evaluate trough concentrations (C_{trough}) of sunitinib and its active metabolite SU12662 using a validated high- performance liquid chromatography–tandem mass spectrometry method (Bioanalytical Systems Inc., West Lafayette, Indiana, USA). An exploratory analysis investigated potential differences in steady-state C_{trough} values of sunitinib, SU12662, and total drug (sunitinib plus SU12662) in Japanese versus non-Japanese patient populations and in patients with different tumor types. C_{trough} from this Japanese study, a Western pancreatic NET trial [15], and from studies of Japanese patients with GIST or RCC [21, 22] were dose-corrected to 37.5 mg and compared.

Blood samples were obtained at screening, week 5, and week 9, and then every 8 weeks to assess chromogranin A (CgA) levels (all patients) and hormone levels (patients with functional tumors only). Patients were required to fast for ≥10 h prior to each scheduled visit. In an exploratory analysis of CgA levels, a biochemical response was defined as a ≥50 % decrease in CgA levels among patients with elevated CgA levels at baseline.

Statistical methods

As pancreatic NETs are rare, a target sample size of at least 10 patients was determined based on feasibility of study conduct rather than statistical requirements. All enrolled patients who received at least one dose of study treatment were included in the efficacy and safety analyses.

Descriptive statistics were used to summarize patient characteristics, treatment administration/compliance, safety parameters, and PK variables. For the analysis of the primary endpoint, the CBR and its exact 95 % CI were calculated. For the analysis of the secondary endpoints, the ORR and its exact 95 % CI, and the percentage change from baseline in the sum of the longest diameter of target lesions were calculated. Time-to-event endpoints (PFS and OS) were summarized using Kaplan–Meier methods.

Results

Patients and treatment

Between July and December 2010, 12 patients (8 male, 4 female) were enrolled in the study at four centers in Japan. All patients received treatment and were analyzed for efficacy and safety. At data cut-off (March 2012), treatment was ongoing in 5 patients with a PR (n=4) or SD (n=1), and 7 patients had withdrawn from the trial. Study withdrawals were due to tumor progression or recurrence (n=3), withdrawal of consent (n=1), treatment interruption >4 weeks due to a serious adverse event (SAE; grade 4 enterocolitis; n=1), and SAEs (grade 4 convulsion plus grade 4 loss of consciousness; n=1). Demographic and baseline disease characteristics are presented in Table 1. All of the patients had well-differentiated pancreatic NETs, of which 10 were classified as nonfunctional and 2 as functional (both gastrinomas). Six patients had received prior octreotide treatment and continued octreotide therapy during the study.

The median relative sunitinib dose intensity was 51 % (range, 26–94); the median number of treatment cycles started was 16 (range, 3–21; Table 2). The sunitinib dose was interrupted in 11 patients and reduced in 8 patients. The most frequently reported cause of dosing interruptions or reductions was AEs.

Efficacy

Based on investigator assessments, 6 of the 12 patients experienced a PR, and none had a CR (Fig. 1). SD ≥24 weeks was observed in 3 patients, and the CBR was 75 % (95 % CI, 43–94). In total, 5/6 patients with prior or concurrent octreotide treatment and 4/6 patients who did not receive octreotide met the criteria for experiencing clinical

Table 1 Patient characteristics at baseline

Patient characteristic	Sunitinib (N=12)
Age, years	
Median	54
Range	34–79
Sex, *n* (%)	
Male	8 (67)
Female	4 (33)
ECOG performance status, *n* (%)	
0	11 (92)
1	1 (8)
Time since diagnosis, years	
Median	3
Range	0.2–9.0
Tumor functionality, *n* (%)	
Nonfunctioning	10 (83)
Functioning	2 (17)
Gastrinoma	2 (17)
Number of involved disease sites per patient, *n* (%)	
1 site	4 (33)
2 sites	5 (42)
3 sites	2 (17)
4 sites	1 (8)
Presence of distant metastases, *n* (%)	
Any, including hepatic	12 (100)
Extrahepatic	3 (25)
Involved disease sites, *n* (%)	
Liver	12 (100)
Lymph node	4 (33)
Pancreas	4 (33)
Lung	2 (17)
Bone	1 (8)
Peritoneum	1 (8)
Prior surgery, *n* (%)	
Yes	9 (75)
No	3 (25)
Prior radiation therapy, *n* (%)	
Yes	1 (8)
No	11 (92)
Number of prior systemic chemotherapy regimens, *n* (%)	
0	6 (50)
1	4 (33)
2	0
≥3	2 (17)

ECOG Eastern cooperative oncology group

benefit. The overall ORR was 50 % (95 % CI, 21–79; Fig. 1). One patient showed a 100 % decrease in target lesion size. One PR occurred in a patient with gastrinoma and was accompanied by a 93 % decrease in plasma gastrin levels (Patient D; Data Supplement Table S1; see also below). In 11/12 patients, some degree of tumor shrinkage was observed by the first assessment 1 month after initiation of sunitinib treatment (Fig. 2). At the time of data cut-off, ongoing patients had been observed for at least 16.1 months, and the median duration of treatment with sunitinib was 9.8 months (range, 0.7–18.1). Although 4/12 patients had discontinued treatment with sunitinib due to reasons other than progressive disease (PD), median PFS had not yet been reached. Six-month and 12-month PFS probabilities were 91 % (95 % CI, 54–99) and 71 % (95 % CI, 34–90), respectively. Median OS had not yet been reached. One death occurred (due to progression of primary disease) during survival follow-up, more than 28 days after the end of treatment.

Table 2 Sunitinib treatment

	Sunitinib (N=12)
Treatment cycles started, median (range)	16 (3–21)
Months on treatment, median (range)	10 (0.7–18)
Months on study, median (range)	14 (0.7–19)
No. of patients with ≥1 dosing interruption, *n* (%)	11 (92)
No. of patients with ≥1 dose reduction, *n* (%)	8 (67)
Relative dose intensity, median (range), %	51 (26–94)

Safety

As of March 2012, the most common treatment-emergent (all-causality) AEs of any grade were diarrhea (n=10; 83 %), hand–foot syndrome and hypertension (both n=8; 67 %); fatigue and headache (both n=7; 58 %), and neutropenia (n=6; 50 %; Table 3). Grade 3 AEs reported in at least 2 patients were neutropenia (n=6; 50 %) and leukopenia (n=2; 17 %). Four patients (33 %) experienced grade 4 AEs, all of which were judged to be related to treatment (herpes encephalitis, convulsion, and loss of consciousness [n=1], increased lipase [n=2], and enterocolitis [n=1]). No deaths related to sunitinib treatment were reported on study or within 28 days of the end of treatment. One death due to disease progression occurred >28 days after end of treatment.

Three patients (25 %) experienced SAEs, all of which resolved. In two cases, the SAEs were assessed as treatment-related. One patient (patient H; Fig. 2) had a grade 4 convulsion and grade 4 loss of consciousness that were reported to be likely due to herpes encephalitis. These SAEs resulted in a sunitinib dose interruption exceeding 4 weeks that led to study discontinuation, as specified in the protocol. Another patient (patient K; Fig. 2) experienced an SAE of grade 4 enterocolitis and temporarily discontinued therapy due to this SAE.

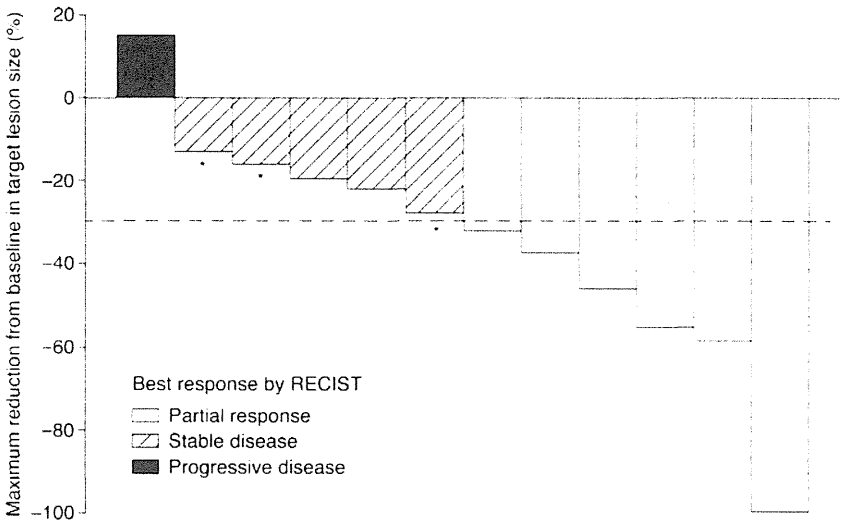


Fig. 1 Maximum percentage reduction from baseline in target lesion size by patient (N= 12). Although one patient had a maximum percentage change in target tumor size from baseline of −100 %, non-target lesions remained and therefore this was not classified as a complete response. *Asterisk* stable disease of ≥24 weeks in duration; *RECIST* Response Evaluation Criteria in Solid Tumors

Pharmacokinetics

Steady-state concentrations of sunitinib, SU12662, and total drug (sunitinib plus SU12662) were reached by day 15 of cycle 1. Subsequent sampling on day 1 of cycles 2–4 showed the concentrations to be sustained following CDD with sunitinib without disproportionate accumulation across cycles (data not shown). Mean dose-corrected (reference dose: 37.5 mg) C_{trough} values were within the ranges of 41.7–53.9 ng/mL for sunitinib, 19.6–25.7 ng/mL for SU12662, and 62.9–77.5 ng/mL for total drug.

We explored potential differences in steady-state C_{trough} values of sunitinib and SU12662 in Japanese versus non-Japanese patient populations and in patients with different tumor types. Dose-corrected steady-state C_{trough} levels from this Japanese study were compared with findings from a Western pancreatic NET population [17], and from studies of Japanese patients with gastrointestinal stromal tumor (GIST) or renal cell carcinoma (RCC) [21, 22]. Steady-state C_{trough} levels of sunitinib, SU12662, or total drug were not significantly different between Japanese and primarily Western patients with pancreatic NET, or between patients with pancreatic NET, GIST, and RCC tumor types (Fig. 3).

Biomarkers

Chromogranin A

Plasma CgA levels were measured in all 12 patients (Fig. 2). At baseline, the median CgA concentration was 9 pmol/mL (range, 3–86 pmol/mL). Six patients had above-median CgA levels at baseline, 3 of whom had a maximum percentage decrease in CgA concentrations of at least −50 %. Among these 3 patients, 2 had a PR and 1 had a best overall response of SD. In the patient with SD, the maximum percentage change in tumor size from baseline was −28 %. Among the 6 patients with below-median CgA levels, 3 experienced a PR and 3 had a best overall response of SD. Tumor responses in all 12 patients did not appear to correlate with the maximum percentage decrease in CgA levels.

Gastrin

Plasma gastrin levels were assessed in the 2 patients with gastrinomas: a 40-year-old female (patient D) and a 34-year-old male (patient L; Data Supplement Table S1). In addition, the relationship between hormonal levels, tumor size, and objective tumor response (based on investigator assessment) was examined in an exploratory analysis. In the male patient, neither gastrin levels nor tumor size decreased after treatment with sunitinib. The best objective response was PD, and the patient discontinued the study at day 79 due to lack of efficacy. In the female patient with gastrinoma, decreases in both gastrin levels (−85 % to −93 %) and tumor size (−31 % to −45 %) were observed during treatment. This patient had a PR on cycle 2 day 1 that was maintained through cycle 7 day 1.

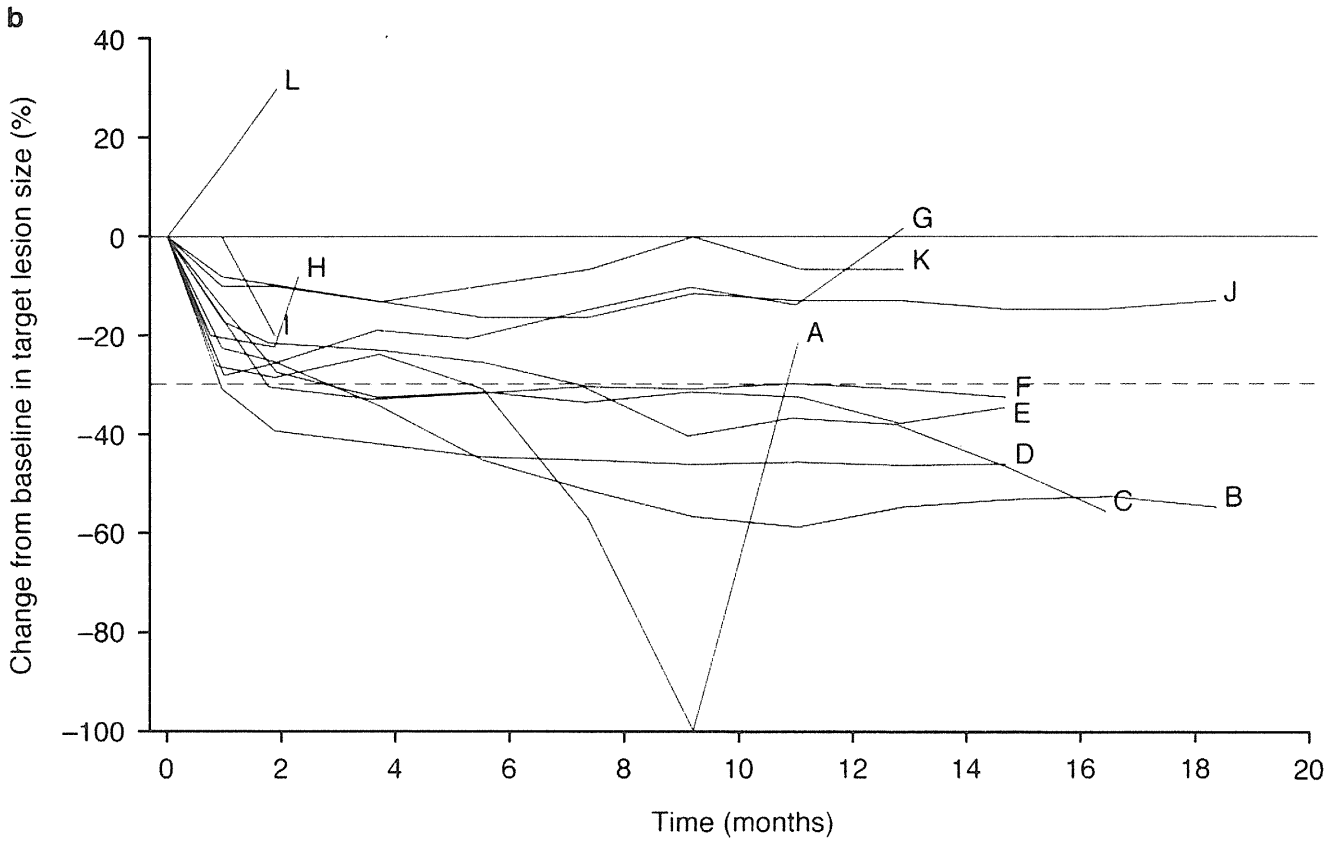
Discussion

This is the first report of sunitinib safety, PK profiles, PFS, and antitumor activity in Japanese patients with unresectable, advanced/metastatic well-differentiated pancreatic NET. While the data are limited by the small sample size, antitumor activity was observed in this population, with a CBR of 75 % and an ORR of 50 %. The ORR was encouraging and higher than the 9 % ORR reported for sunitinib in a randomized, phase III trial in a predominantly non-Asian population [7]. In the present study, 11 patients had

a

Patient	Age, sex	Tumor functionality	Prior treatment	Maximum change in target lesion size (%)[a]	Best overall response	PFS (months)	Reason for discontinuation	Chromogranin A	
								Baseline concentration (pmol/mL)	Maximum change from baseline (%)[a]
A	62, F	Non-functioning	Gemcitabine, somatostatin analogs	−100	PR	11.1	PD	4	5
B	44, M	Non-functioning	Epirubicin, mitomycin-C	−59	PR	18.6[b]	None – ongoing	3	62
C	64, F	Non-functioning	Epirubicin, somatostatin analogs	−56	PR	16.6[b]	None – ongoing	13	−53
D	40, F	Functioning (gastrinoma)	Somatostatin analogs	−46	PR	15.0[c]	None – ongoing	86	−89
E	64, M	Non-functioning	None	−38	PR	14.8	PD	4	47
F	51, M	Non-functioning	Cisplatin, etoposide, somatostatin analogs	−33	PR	14.8[c]	None – ongoing	10	−5
G	46, M	Non-functioning	Fluorouracil, cisplatin, gemcitabine, streptozocin, tegafur-uracil, somatostatin analogs	−28	SD	9.3	PD	18	−73
H	57, M	Non-functioning	Somatostatin analogs	−22	SD	2.4[b]	Treatment interruption >4 consecutive weeks due to lack of tolerance (serious adverse events of convulsion and loss of consciousness)	7	−37
I	64, F	Non-functioning	None	−20	SD	2.1[b]	Withdrawal of informed consent	16	−1
J	44, M	Non-functioning	Epirubicin, cisplatin, cancer vaccinations, cyclophosphamide	−16	SD	18.5[b]	None – ongoing	6	−19
K	79, M	Non-functioning	None	−13	SD	13.0[b]	Serious adverse event (grade 4 enterocolitis)	8	−29
L	34, M	Functioning (gastrinoma)	None	15	PD	2.0	PD	21	300

b



◀ **Fig. 2** Individual patient profiles and response to treatment (N=12). **a** Summary of patient profiles and changes in tumor-size and chromogranin A levels. **b** Percentage change from baseline in target lesion size over time in individual patients. [a] Maximum % change = [(minimum value after dosing – baseline)/baseline] x 100; [b] Based on censored data; *F* female; *M* male; *PD* progressive disease; *PFS* progression-free survival; *PR* partial response; *SD* stable disease

decreases in target lesion measurements and achieved a best response of a PR or SD. Analysis of the percentage change in target lesion size over time showed a trend in tumor shrinkage from the first assessment 1 month after initiation of treatment with sunitinib. As of March 2012, ongoing patients had been observed for at least 16.1 months, and median PFS had not been reached. The probability of being alive and progression-free at 6 months was 91 % (95 % CI, 54–99) and at 12 months was 71 % (95 % CI, 34–90). In the randomized sunitinib phase III trial, median PFS was 11.4 months [7]. These data suggest that PFS in Japanese patients receiving sunitinib in our study may be equivalent to or greater than that observed in the randomized phase III trial.

The potential effect of octreotide therapy was difficult to evaluate in this study due to the small sample size. The CBR was similar among patients with (n=5/6) or without (n=4/6) octreotide treatment. In an exploratory subpopulation analysis reported in the randomized sunitinib phase III study, the efficacy of sunitinib appeared similar in patients who did and did not receive somatostatin analogues (PFS hazard ratios of 0.43 and 0.41, respectively), suggesting that the efficacy of sunitinib was not affected by somatostatin analogue treatment [7].

AEs reported during sunitinib treatment were manageable with palliative care measures, such as dosing interruption. The AEs observed in this study were similar to those reported in a phase III study in primarily Western patients with pancreatic NET [7], and frequently observed AEs were comparable to those reported in patients with GIST or RCC [21, 22].

In the current study, the median number of treatment cycles started was 16. All patients had grade 3/4 AEs and at least one sunitinib dosing interruption. However, the therapeutic effect of sunitinib did not appear to be reduced by temporary dosing interruptions due to AEs. Neutropenia, the most common grade 3/4 AE, was also the most frequently reported grade 3/4 toxicity in the predominantly Western sunitinib phase III pancreatic NET study [7], although the frequency of this event was markedly higher in our study with Japanese patients (42 % vs. 12 %). Increased rates of grade 3/4 neutropenia have also been observed in sunitinib-treated Japanese patients with GIST (37 % vs. 10 %) or RCC (53 % vs. 18 %), compared with predominantly Western populations [21–24]. The frequency of grade 3/4 thrombocytopenia similarly appeared to be higher in sunitinib-treated Japanese vs. Western patients (GIST: 20 % vs. 4 %; RCC: 55 % vs. 9 %; pancreatic NET:

Table 3 Treatment-emergent (all-causality) adverse events (AEs) reported in ≥25 % of patients, according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0

	Maximum grade (G), *n* (%)			
AE	G1	G2	G3	Total[a]
Any AE[b]	0	0	8	12 (100)
Diarrhea	4 (33)	5 (42)	1 (8)	10 (83)
Hand–foot syndrome	1 (8)	7 (58)	0	8 (67)
Hypertension	1 (8)	7 (58)	0	8 (67)
Fatigue	1 (8)	6 (50)	0	7 (58)
Headache	4 (33)	3 (25)	0	7 (58)
Neutropenia	0	0	6 (50)	6 (50)
Dysgeusia	5 (42)	0	0	5 (42)
Nasopharyngitis	4 (33)	1 (8)	0	5 (42)
Nausea	4 (33)	1 (8)	0	5 (42)
Pyrexia	2 (17)	2 (17)	1 (8)	5 (42)
Vomiting	5 (42)	0	0	5 (42)
Decreased appetite	4 (33)	0	0	4 (33)
Edema	3 (25)	1 (8)	0	4 (33)
Hypothyroidism	0	4 (33)	0	4 (33)
Leukopenia	0	1 (8)	2 (17)	3 (25)
Mucosal inflammation	2 (17)	1 (8)	0	3 (25)
Muscle spasms	3 (25)	0	0	3 (25)
Prolonged electrocardiogram QT	1 (8)	1 (8)	1 (8)	3 (25)
Thrombocytopenia	1 (8)	1 (8)	1 (8)	3 (25)

[a]Grade 4 AEs were observed in 4 patients: convulsion, loss of consciousness, and herpes encephalitis (n=1), increased lipase (n=2), and enterocolitis (n=1); no grade 5 AEs were reported

[b]Patients were counted once, with only the highest grade AE listed

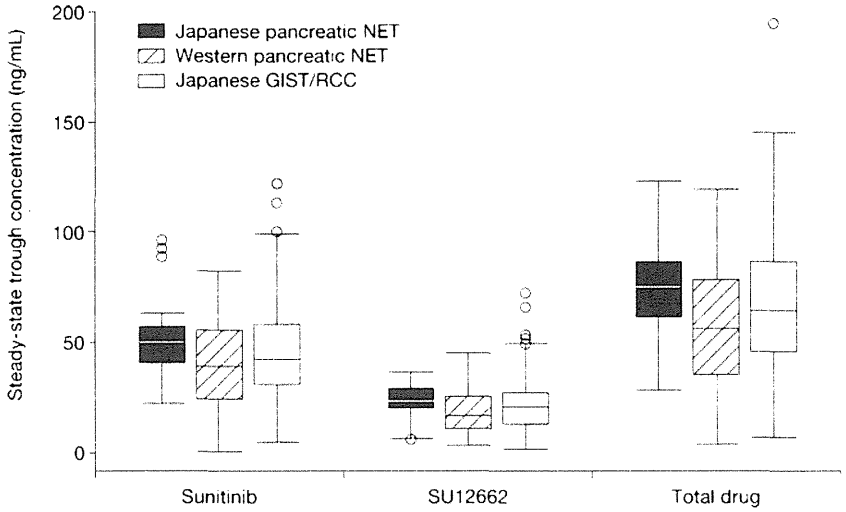


Fig. 3 Trough concentrations of sunitinib, active metabolite SU12662, and total drug (sunitinib plus SU12662) in Japanese patients with pancreatic neuroendocrine tumor (NET; $n=11$; present study) or gastrointestinal stromal tumor (GIST; $n=30$) [21] and renal cell carcinoma (RCC; $n=38$; pooled data) [25], and in predominantly Western patients with pancreatic NET; $n=57$ [17]. The sunitinib dose in each study was dose-corrected to 37.5 mg. The *upper* and *lower box boundaries* denote the 75th and 25th percentiles, respectively, with the median shown as a line within the box. *Whiskers* indicate minimum and maximum values. Outlying values are denoted as *circles*

17 % vs. 4 %) [21–24]. It should be noted that the sunitinib GIST and RCC trials used a different dosing schedule (sunitinib 50 mg/day, for 4 weeks on therapy, followed by 2 weeks off) than our study and the phase III, pancreatic NET trial.

It is not clear why rates of grade 3/4 hematologic AEs appear to be higher in Japanese versus Western patients who receive sunitinib. Analysis of PK parameters has shown that the area under the concentration–time values of sunitinib and SU12662 are similar in Japanese and Caucasian patients with RCC [22]. In addition, when steady-state C_{trough} values from this Japanese study were compared with those from a Western pancreatic NET population [17] and with C_{trough} values from Japanese patients with GIST or RCC [21, 22], there were no significant differences in the dose-corrected C_{trough} levels of sunitinib, SU12662, or total drug between Japanese and primarily Western patients with pancreatic NET, or among patients with pancreatic NET, GIST, or RCC. In the absence of racial or ethnic differences in the PK of sunitinib, Uemura et al. [25] suggested that the elevated rates of grade 3/4 hematologic AEs in Japanese patients may be due to differences in the expression levels and activity of sunitinib-sensitive kinases involved in the regulation of hematopoiesis.

Everolimus, an inhibitor of the mammalian target of rapamycin, is approved for the treatment of pancreatic NET in Japan, and like sunitinib is commonly associated with skin and gastrointestinal disorders [9]. Additional AEs related to sunitinib treatment include hematotoxicity, cardiovascular disorders and constitutional symptoms [26], while pneumonitis and infections are associated with everolimus therapy [27]. These different safety profiles reflect each compound's distinct mode of action. No racial differences between Japanese and Western patients have been reported for the safety profile of either drug, based on the current study and a subgroup analysis of Japanese patients in the RADIANT-3 everolimus trial [9].

Treatment-emergent changes in CgA levels may provide a means to select patients with pancreatic NET likely to benefit from molecular targeted therapy [28]. However, in this study tumor responses in all 12 patients did not appear to correlate with the maximum percentage decrease in CgA levels, possibly because of small patient numbers with elevated CgA concentrations at baseline. Patient D had the highest baseline CgA levels in the study (86 pmol/mL), and decreased CgA concentrations (−89 %) were subsequently observed in combination with a PR. An increase in CgA levels (300 %) occurred during the study in 1 patient (Patient L) who experienced PD. A potential correlation between changes in CgA levels and clinical benefit was considered in these 2 patients. In patients with elevated baseline CgA concentrations, CgA appeared to be a useful marker in patients with pancreatic NET as reported previously [29].

The use of sunitinib marks a new phase in the development of a more targeted approach to the treatment of advanced-stage pancreatic NET. Results from the current study demonstrate antitumor activity in Japanese patients with unresectable, well-differentiated pancreatic NET and corroborate earlier findings in Western and Asian populations.

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Conflict of interest Richard Chao, Satoshi Hashigaki, Nobuyuki Kimura, and Emiko Ohki are employees of Pfizer, and N. Kimura, E. Ohki, and R. Chao hold Pfizer stock. Mami Murakami was previously employed by Pfizer. Tetsuhide Ito, Takuji Okusaka and Kenji Yamao have received research funding from Pfizer. Toshirou Nishida has received research funding from Pfizer and Novartis Pharmaceuticals. Hisato Igarashi, Nobumasa Mizuno, Kazuo Hara, Chigusa Morizane, Shunsuke Kondo, Akira Sawaki, and Masayuki Imamura have no potential conflicts of interest to disclose.

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Original Article

BCL10 as a useful marker for pancreatic acinar cell carcinoma, especially using endoscopic ultrasound cytology specimens

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Acinar cell carcinomas (ACCs) of the pancreas are characterized by the histological and immunohistochemical features of acinar cell differentiation. Recently, BCL10, originally identified as a recurrent t(1;14)(p22;q32) translocation in MALT B-cell lymphoma, was found to be immunohistochemically positive in some solid tumors, including ACC. To evaluate its diagnostic efficacy, we performed BCL10 immunohistochemistry and evaluated molecular markers correlated to pancreatic tumor lineages (neuroendocrine markers and a mutation analysis of *KRAS* and *GNAS*) using samples from 126 pancreatic tumors (17 ACCs, 24 pancreatic ductal adenocarcinomas, 4 adenosquamous carcinomas, 9 intraductal papillary mucinous neoplasms, 10 mucinous cystic neoplasms, 44 neuroendocrine tumors, 9 serous cystic tumors and 10 solid-pseudopapillary neoplasms). BCL10 was exclusively expressed in normal acini. In pancreatic tumors, 14 of 17 (82%) ACCs and 2 of 4 (50%) adenosquamous carcinomas were positive, while the other subtypes were almost negative. We subsequently examined the diagnostic utility of BCL10 in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimens using 57 pancreatic tumors. BLC10 correctly identified ACCs (9/13) and adenosquamous carcinomas (2/4) but none of the other subtypes (n = 41). Therefore, we suggested that BCL10 expression is a useful marker for acinar cell differentiation, particularly in the diagnosis of EUS-FNA specimens.

Key words: acinar cell carcinoma, BCL10, differential diagnosis, immunohistochemistry, pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDA) is a major subtype of pancreatic cancer that is followed by neuroendocrine tumors. These two major subtypes represent the cellular composition of the pancreas. Although most other neoplasms are associated with ductal epithelial differentiation, i.e. intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), some tumors show acinar features. Pancreatic acinar cell carcinoma (ACC) is a tumor characterized by acinar cell features and comprises less than 2% of all pancreatic neoplasms.[1,2] This subtype is clinically and pathologically distinct from typical PDAs. The median age of the patients with ACC is slightly older than PDA, and ACC shows a better prognosis despite more frequent distant metastasis. Histologically, ACC has characteristic acinar differentiation that is illustrated by immunohistochemical staining for trypsin, chymotrypsin, and/or lipase.[3–5] Furthermore, ACCs have been reported to lack a *KRAS* mutation, suggesting that they develop via different molecular pathways than PDAs. Abnormalities in tumor suppressor genes, such as *TP53*, *DPC4/Smad4* and *p16*, are less common than in PDAs.[3,6–9]

BCL10 was recently identified through the cloning of a (1;14)(p22;q32) translocation breakpoint in several cases of low-grade mucosa-associated lymphoid tissue (MALT) B-cell lymphoma.[10,11] This gene, localized to chromosome band 1p22, is a cellular homolog of the equine herpesvirus-2 E10 gene; both contain an amino-terminal caspase recruitment domain (CARD) homologous to that found in several apoptotic molecules.[12] Mutation analyses of BCL10 have implicated this gene in MALT lymphomas and other lymphoid tumors of the B- or T-cell lineage without t(1;14)(p22;q32) translocation.[13–15] In addition, BCL10 abnormalities have been reported in solid cancer cell lines and tumors, including malignant mesotheliomas, germ cell tumors, and colon carcinomas, suggesting that BCL10 can contribute to the pathogenesis of several types of neoplasia.[10,16] In contrast to a number of articles about BCL10 expression in lymphoid

malignancies, only a limited number of articles have documented its expression in solid cancers. Chang *et al.* have reported that BCL10 expression is significantly associated with the progression and prognosis of oral squamous cell carcinomas, while BCL10 has been reported by Kuo *et al.* to play an important role in controlling the growth of cervical cancer cells through NF-κB-dependent cyclin D1 regulation.[17,18] In the pancreas, La Rosa *et al.* recently reported that BCL10 was expressed specifically in acinar cells and acinar cell carcinomas.[5,19]

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was introduced into clinical practice in the early 1990s and is now considered one of the most useful methods for the histological diagnosis and staging of pancreatic cancers.[20] However, specimens obtained by EUS-FNA are tiny and fragmented, and a definitive diagnosis is frequently challenging for pathologists. We recently reported a diagnostic scheme for EUS-FNA specimens of three major pancreatic tumor types using a minimal number of markers, including CK7, CDX2, synaptophysin, chromogranin A and *KRAS* mutations.[21] In that study, ACC was characterized by occasional expression of CK7 and CDX2, lack of a *KRAS* mutation, and various expression patterns of neuroendocrine markers. When heterogeneous expression and staining errors were considered, it was determined that some positive markers were required in this panel. In the present study, we found that BCL10 is expressed exclusively in ACC, implying that it could serve as a useful marker for labeling this rare, well-differentiated subtype, particularly when diagnosing EUS-FNA specimens.

MATERIALS AND METHODS

Patients and tissues

A total of 126 pancreatic tumors from 124 patients were selected from the database of the Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Nagoya, Japan. This cohort was composed of 17 ACCs, 23 PDAs, 4 adenosquamous carcinomas, 9 IPMNs, 10 MCNs, 44 neuroendocrine tumors (including 41 well-differentiated tumors and three poorly differentiated tumors, according to the WHO classification[2]), 9 serous cystic tumors and 10 solid-pseudopapillary neoplasms. Of these, surgical materials were available in 116 tumors. EUS-FNA procedures were performed in 58 tumors, of which 48 underwent subsequent surgical procedures. All specimens were fixed with formalin and embedded in paraffin. Aspirates were also fixed with formalin and then processed to make cell blocks. This study was a part of a comprehensive research program of the tissue bank in Aichi Cancer Center that had been approved by an institutional review board.

Immunohistochemistry

Immunohistochemistry was performed using an Autostainer Link 48 (Dako, Copenhagen, Denmark) according to the manufacturer's instructions. Anti-BCL10 mouse monoclonal antibody (Clone 331.3; Santa Cruz, San Francisco, CA, USA) was used at a 1200-fold dilution, and anti-trypsin mouse monoclonal antibody (Clone 430; Biodesign/Meridian Life Science, Memphis, TN, USA) was used at a 1000-fold dilution. The antigens were retrieved by PT Link (Dako) for 30 min in a High Buffer Solution (pH 9.0, Dako). The staining patterns of positive BCL10 and trypsin reactions were classified into the four-tiered scoring system: negative; 1+, faint and focal staining (in less than 50% of the total area); 2+, faint but diffuse staining (in more than 50% of the total area) or strong but focal staining (in less than 50% of the total area); and 3+, strong and diffuse staining (in more than 50% of the total area). We evaluated the staining as positive when the tumor cells showed moderate or greater intensity in 50% of the area (2+, or 3+). Detection of synaptophysin and chromogranin A expression has been reported elsewhere.[21]

Mutation analysis

The *KRAS* mutation status of the individual tumors was obtained from our database, and the details of the methods used to detect the mutations have been previously reported.[21,22] For the detection of the *GNAS* mutation, we developed a sensitive method using a cycleave PCR technique similar to that for *KRAS* mutation detection. The details are reported elsewhere (personal communication).

Statistical analysis

Fisher's exact test, a χ^2 test for independence and an unpaired *t*-test were used to compare gene expression between histological subtypes. Logistic regression models were constructed to analyze more complex relationships using the SYSTAT software (SYSTAT Software Inc., Richmond, CA, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

BCL10 expression of the normal pancreas and pancreatic cancers

We first examined the expression pattern of BCL10 in normal pancreatic tissue. As shown in Fig. 1, BCL10 expression was observed exclusively in the cytoplasm of acinar cells. This

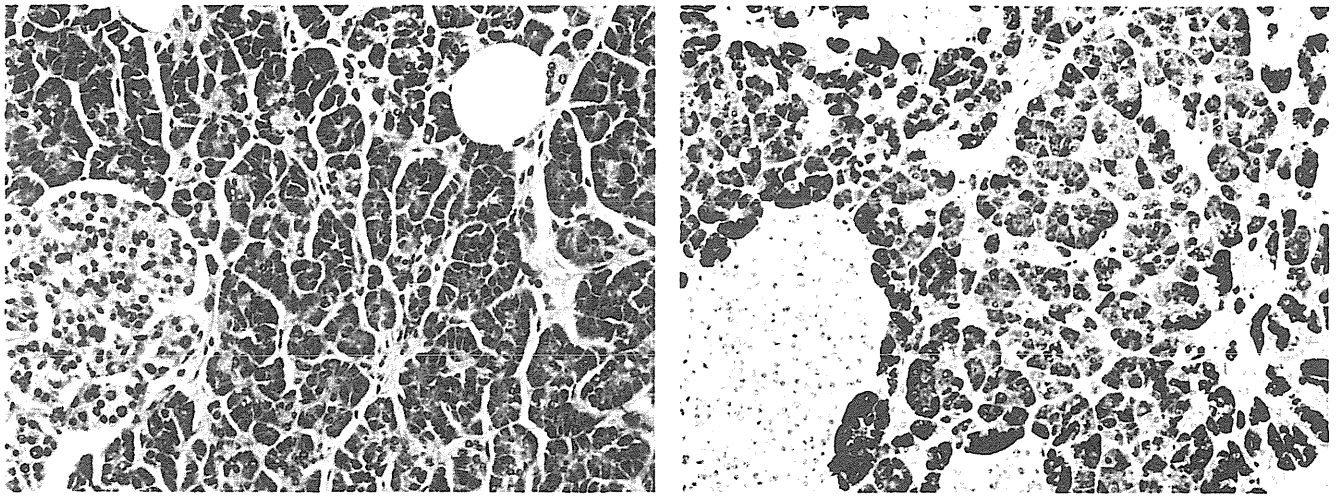

Figure 1 BCL10 expression in the normal pancreas. BCL10 uniformly labels acinar cells but not islet cells.

pattern was highly contrasted to those of CDX2 and CK7 in the ductal epithelial cells from the centroacinar cells to the main pancreatic ducts and the centroacinar cells to the intercalated ducts, respectively, as reported previously.[21] No other cells were positive for BCL10, suggesting that BCL10 could serve as a good marker for acinar cells.

This restricted pattern was also present in the pancreatic cancer tissues. We examined a wide range of pancreatic tumors in which intensely and diffusely positive reactions were detected in the majority of ACCs (14/17, 82%) (Fig. 2). Two of four adenosquamous carcinomas were positive, and the reaction was notably restricted to carcinoma cells that showed distinct keratinocytic differentiation (Fig. 3a,b). Other subtypes of pancreatic tumors were mostly negative (Table 1).

Lack of *KRAS* and *GNAS* mutations in BCL10-expressing cancers (Table 1)

Some gene mutations occur in a cancer type-specific manner. It has been shown that *KRAS* is mutated exclusively in PDAs, IPMNs and MCNs, while the *GNAS* mutation has been reported to be specific to IPMNs.[23–27] Therefore, we examined whether BCL10 expression is related to these cancer type-specific mutations and neuroendocrine markers of neuroendocrine tumors. BCL10 was positive in three of 30 *KRAS*-mutated tumors, and they were histologically classified as adenosquamous carcinoma (two cases) and IPMN (one case). *GNAS* mutations were found in 3 of 114 tumors, all of which were diagnosed as IPMN. No neuroendocrine tumors were positive for BCL10. PDAs, IPMNs and neuroendocrine tumors account for 92% of pancreatic cancers,[1] and BCL10 was not expressed in these major pancreatic cancers. This result genotypically supported the ACC-specific expression of BCL10.

Specific BCL10 staining in ACC in EUS-FNA samples (n = 13, Table 2)

Currently, EUS-FNA is the standard in the histological diagnosis of pancreatic cancers. However, specimens obtained by this method are tiny and fragmented, and a definitive diagnosis is frequently challenging for pathologists. Therefore, we examined whether BCL10 expression could be used as a differential immunohistochemical marker in EUS-FNA samples. BCL10 was labeled in nine of 13 ACCs, whereas none of the other 43 tumors, except two adenosquamous carcinomas, were positive for BCL10 (Table 2, Fig. 3c,d).

DISCUSSION

Various cellular components are present in the pancreas, and the current classification of pancreatic cancer is based on the knowledge of such normal counterparts. ACC is thought to be derived from pancreatic acinar cells, and ACC mimics the morphology and phenotype of the normal counterpart, acinar cells, including trypsin expression. However, ACC is frequently positive for neuroendocrine makers, despite early divergence in the development of the pancreas.[28] As shown in Tables 1 and 2, approximately one third of ACCs expressed neuroendocrine markers. Furthermore, both tumors shared morphological characteristics including

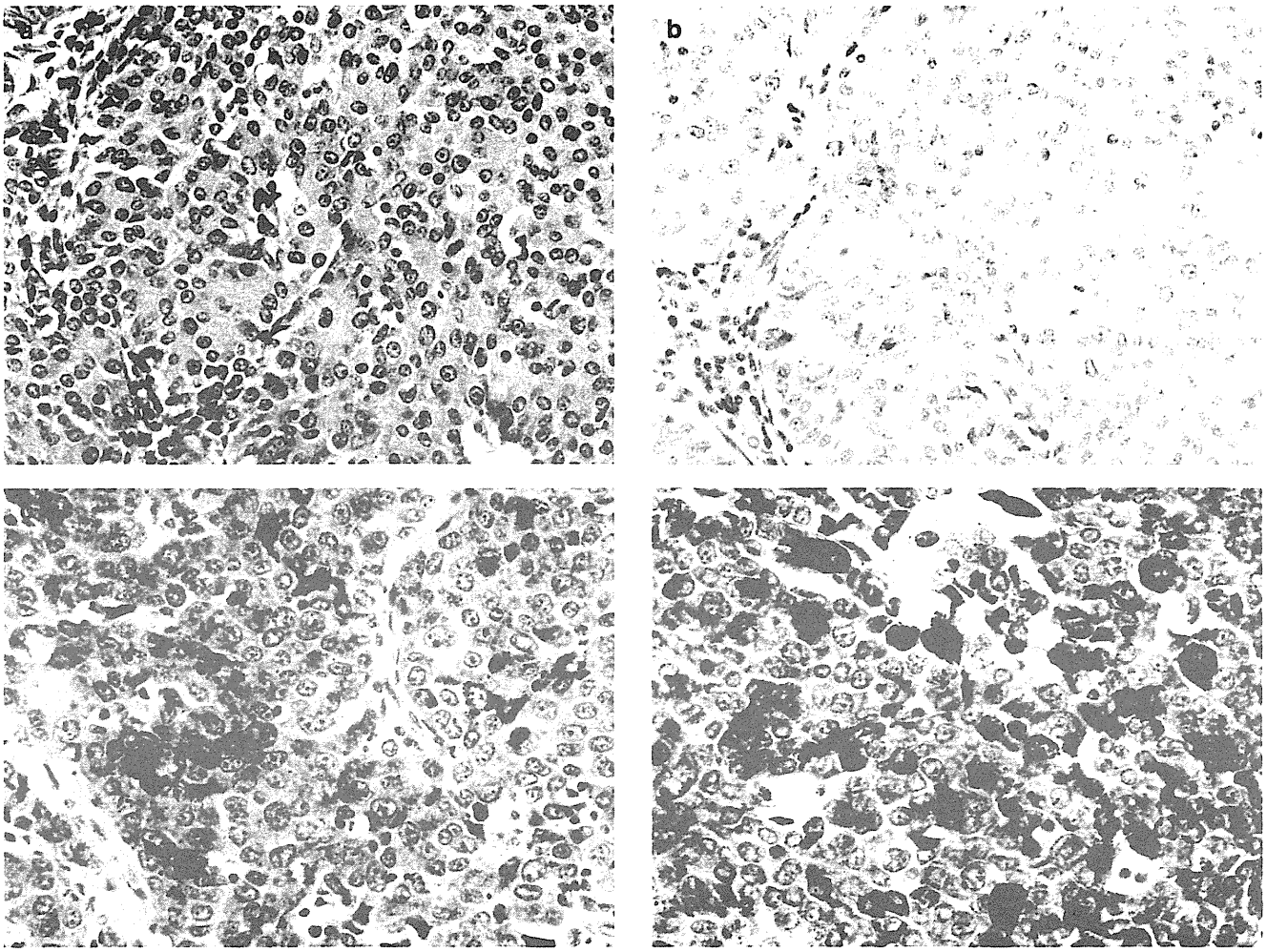

Figure 2 Typical acinar cell carcinoma. (**a**) An acinar cell carcinoma showing small acinar-like nests composed of small round nuclei and ample basophilic cytoplasms. Focal positive reactions for (**b**) synaptophysin and (**c**) trypsin were observed. (**d**) Despite divergence of the intensity, most tumor cells were positive for BCL10.

small round nuclei, central localization of the nuclei in the cytoplasm, relative ample cytoplasm, and sheet-like growth. These morphological characteristics, in addition to the frequent expression of neuroendocrine markers, make the differential diagnosis between ACC and neuroendocrine tumor difficult. However, this study clearly demonstrated that BCL10 could be used in this setting. None of the 44 neuroendocrine tumors, which included three cases of poorly differentiated neuroendocrine carcinomas, were positive for BCL10, but 82% (14/17) of ACCs expressed BCL10. This specific expression is especially useful for EUS-FNA samples because only a tiny piece of the tissues is allowed to be examined, and in many cases, no normal acini are included for control staining. Indeed, the commonly used ACC markers, such as trypsin and chymotrypsin, sometimes stained weakly and focally. Even though overall frequency of positive trypsin was higher than that of BCL10, distinctively positive reactions (2+ and 3+ in Table 2) were limited to only one third of ACCs. Furthermore, faint reaction (1+) in two surgical specimens was not detected in the EUS-FNA samples. This is in sharp contrast to BCL10, in which we found clear labeling (all 2+ or 3+) in EUS-FNA samples of ACCs. Such clear reaction is particularly crucial for diagnosis with EUS-FNA samples. Although two articles from the same group have reported specific expression of BCL10 in ACC using surgical specimens,[5,19] we confirmed the finding with detailed genotypes, and found that the expression was useful particularly in diagnosis using EUS-FNA samples.

In addition to neuroendocrine tumors, ACC with prominent ductal differentiation may be problematic in differential diagnoses. Stelow *et al.* have recently reported 11 such cases, five and six of which showed some morphological features of mucinous carcinoma and typical ductal carcinoma, respectively.[29] For this type of tumor, a differential diagnosis with

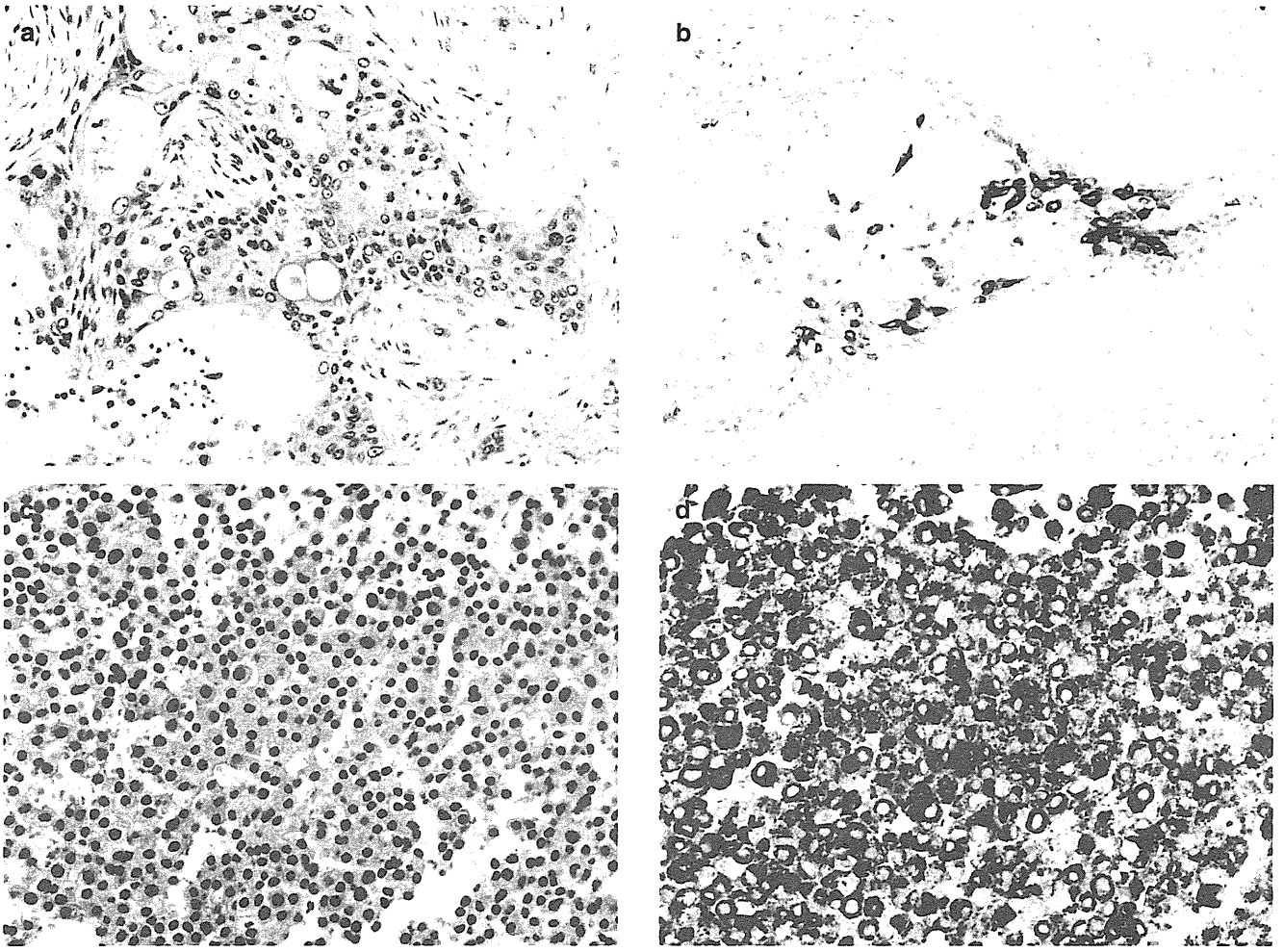

Figure 3 (**a**,**b**) Adenosquamous carcinoma and (**c**,**d**) an EUS-FNA specimen of an acinar cell carcinoma. Some pancreatic cancers showed differentiation to both glandular and squamous cells, and these tumors were classified as adenosquamous carcinoma. (**b**) With immunohistochemical staining of BCL10, squamous but not adenocarcinomatous components were positive. The lower panel is a representative case of acinar cell carcinoma in an EUS-FNA sample. (**c**) Only a limited number of tumor cells were obtained, and the acinar cell carcinoma shared morphological characteristics with neuroendocrine tumors. Thus, the differential diagnosis is challenging in cases such as this. However, (**d**) the specific expression of BCL10 in acinar cell carcinoma is quite helpful.

Table 1 Expression of BCL10 and neuroendocrine markers and genetic analyses of the *KRAS* and *GNAS* genes in 126 pancreatic tumors

		BCL-10								
		Positive		Negative			*KRAS*	*GNAS*	CGA	SYN
Subtype	Total (*n*)	3+	2+	1+	0	Positive rate (%)	Mutation rate (%)	Mutation rate (%)	Positive (%)	Positive (%)
ACC	17	10	4		3	14/17 (82%)	0/16 (0%)	0/16 (0%)	6/17 (35%)	8/17 (47%)
ADSQ	4		2	1	0	2/4 (50%)	4/4 (100%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
PDA	23			1	22	0/23 (0%)	20/22 (91%)	0/21 (0%)	0/20 (0%)	0/20 (0%)
IPMN	9		1	3	5	1/9 (11%)	3/8 (38%)	3/8 (38%)	n.a.	n.a.
MCN	10		1	3	6	1/10 (10%)	1/5 (20%)	0/6 (0%)	n.a.	n.a.
NET	44			1	43	0/44 (0%)	2/44 (5%)	0/43 (0%)	43/44 (98%)	44/44 (100%)
SCN	9			1	8	0/9 (0%)	0/7 (0%)	0/7 (0%)	n.a.	n.a.
SPN	10		1	2	7	1/10 (10%)	0/9 (0%)	0/9 (0%)	2/7 (29%)	5/7 (71%)

ACC, acinar cell carcinoma; ADSQ, adenosquamous carcinoma; CGA, chromogranin A; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; n.a., not assessed; NET, neuroendocrine tumor; PDA, pancreatic ductal adenocarcinoma; SCN, serous cystic neoplasm; SPN, solid pseudopapillary neoplasm; SYN, synaptophysin.

Table 2 Results of the immunohistochemical and genetic analyses of 58 pancreatic tumors using EUS-FNA specimens

		EUS-FNA						Resected	
Case ID.	Subtype	*KRAS* mutation	*GNAS* mutation	CGA	SYN	BCL10	Trypsin	BCL10	Trypsin
1	ACC	WT	WT	+, focal	–	2+	2+	3+	2+
2	ACC	WT	WT	–	–	3+	–	3+	2+
3	ACC	WT	WT	–	–	–	equivocal	2+	2+
4	ACC	WT	WT	–	–	2+	1+	3+	1+
5	ACC	WT	WT	–	+, focal	3+	1+	3+	1+
6	ACC	WT	WT	–	–	–	–	–	1+
7	ACC	WT	WT	–	–	–	–	–	1+
8	Mixed Ductal-ACC	WT	WT	+	+	3+	equivocal	2+	2+
9	ACC	WT	WT	–	–	3+	1+		
10	ACC	WT	WT	+	+	3+	2+		
11	ACC	WT	WT	–	+, focal	2+	3+		
12	ACC	WT	WT	–	+, focal	2+	2+		
13	ACC	WT	WT	+, focal	+, focal	–	1+		
	PDA (n = 18)	15/17 (88%)	0/14 (0%)	0/15 (0%)	0/15 (0%)	0/18 (0%)			
	ADSQ (n = 4)	4/4 (100%)	0/4 (0%)	n.a.	n.a.	2/4 (50%)			
	NET (n = 17)	2/17 (12%)	0/16 (0%)	17/17 (100%)	17/17 (100%)	0/17 (0%)			
	SPN (n = 6)	0/6 (0%)	0/6 (0%)	2/3 (67%)	2/3 (67%)	0/6 (0%)			

Two cases of acinar cell carcinomas showed equivocal results, in which non-specific staining was too pronounced to make a definite evaluation.
ACC, acinar cell carcinoma; ADSQ, adenosquamous carcinoma; CGA, chromogranin A; n.a., not assessed; NET, neuroendocrine tumor; PDA, pancreatic ductal adenocarcinoma; SPN, solid pseudopapillary neoplasm; SYN, synaptophysin; WT, wild-type.

PDA, IPMN and MCN is needed. Fortunately, good genetic markers are available: *KRAS* and *GNAS*. *KRAS* mutations were previously detected in 95% or more of PDAs, 40–80% of IPMNs, and 30% of MCNs, while *GNAS* mutations were specific to IPMNs.[23–27] This study revealed that BCL10 was expressed in a mutually exclusive fashion to *KRAS* and *GNAS* mutations, suggesting that BCL10-expressing tumors are distinct from PDAs, IPMNs and MCNs.

It is also interesting that adenosquamous carcinomas expressed BCL10, although the number of examined samples was limited. Prior to this study, we examined BCL10 expression in 130 tumors of various organs using tissue microarray (data not shown, but could be provided as supplementary data if requested). Five of 33 squamous carcinomas but no adenocarcinomas were positive for BCL10. Indeed, some reports have noted that BCL10 is expressed in most oral squamous cell carcinomas[17,30] and that the intensity is associated with cancer progression and prognosis.[17] Therefore, squamous cell differentiation could be a pitfall of the interpretation of BCL10 expression in pancreatic tumor subtyping.

In summary, we examined BCL10 expression in pancreatic cancer. BCL10 was specifically expressed in ACCs, and none of the BCL10-expressing tumors harbored the *KRAS* or *GNAS* mutations that are frequently mutated in PDAs, IPMNs and MCNs. ACC is often difficult to distinguish from neuroendocrine tumors, particularly when limited samples are obtained, because approximately one third of ACCs are positive for neuroendocrine markers and the two tumors share morphological characteristics. BCL10 could be a useful marker in this setting.

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