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PHASE I STUDIES

Phase I study of resminostat, an HDAC inhibitor, combined with S-1 in patients with pre-treated biliary tract or pancreatic cancer

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Summary

Resminostat is an oral hydroxamate inhibitor of class I, IIb, and IV histone deacetylases. S-1 is widely used to treat biliary tract cancer and pancreatic cancer in Japan. We performed a phase I study of resminostat combined with S-1 as second-line or later therapy in Japanese patients with biliary tract or pancreatic cancer. A total of 27 patients were enrolled. We determined the optimal regimen for resminostat/S-1 therapy in part 1, and investigated its safety and efficacy in part 2. In part 1, 17 patients were enrolled. One DLT (anorexia and stomatitis, respectively) occurred with each of regimens 2 and 3. In part 2, an additional 10 patients received regimen 3, which was selected in part 1. Regimen 3 was resminostat (200 mg/day on Days 1 to 5 and Days 8 to 12: 5 days on/2 days off) plus S-1 (80–120 mg/day according to body surface area on Days 1 to 14) repeated every 21 days. A total of 16 patients (13 with biliary tract cancer and 3 with pancreatic cancer) received regimen 3 and it was well tolerated. The most frequent treatment-related adverse events were thrombocytopenia and anorexia (11 patients each, 69%). The disease control rate was 81.3% (84.6% for biliary tract cancer and 66.7% for pancreatic cancer, respectively). Median progression-free survival was 3.1 months (5.5 and 2.3 months), while median overall survival was 8.8 months (10.2 and 4.7 months). In conclusion, regimen 3 was well tolerated by patients with pre-treated biliary tract or pancreatic cancer.

Keywords Biliary tract cancer · Histone deacetylase · Resminostat · S-1 · Systemic cancer therapy

Introduction

Surgical resection is the only radical treatment available for biliary tract cancer (BTC) and pancreatic cancer (PC), but most tumors are unresectable at the time of diagnosis and a limited number of patients are eligible for curative resection. Even after resection, BTC and PC show recurrence after a short period and the prognosis is extremely poor. Systemic chemotherapy is the first-line treatment in patients with unresectable BTC/PC or with postoperative recurrence. However, current therapeutic regimens are unsatisfactory, which means that development of more effective agents is urgently required [1].

First-line treatment using gemcitabine (GEM) plus cisplatin is regarded as the international standard of care for patients with unresectable or recurrent BTC [2]. Two clinical studies of monotherapy with S-1, an oral fluoropyrimidine, as second-line treatment for BTC have been conducted in Japan. The response rate (RR) was 7.5%, progression-free survival (PFS) was 2.5 months, and median survival time (MST) was 6.8 months in one study, while RR was 22.7%, time to progression was 5.4 months, and MST was 13.5 months in the other study [3, 4]. Although both studies were single-arm

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trials that were not designed to demonstrate a survival benefit, S-1 is now a widely used second-line treatment for BTC in Japan because definite antitumor activity was demonstrated.

For unresectable or recurrent PC, first-line combination chemotherapy using oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX), or nanoparticle albumin-bound paclitaxel (nab-PTX) with GEM is regarded as the international standard of care for patients with a good performance status (PS) [5]. Either GEM or S-1 monotherapy is widely employed in Japan as second-line treatment for PC, since it is considered desirable to select drugs that were not used for first-line therapy [6]. In a phase II study performed in patients with metastatic PC, second-line S-1 monotherapy achieved a median PFS of 2.0 months and MST of 4.5 months [7].

Resminostat is an oral anticancer agent that inhibits histone deacetylases (HDACs) of classes I, IIB, and IV. HDAC are involved in epigenetic regulation by acting on histones in nucleosomes to modify chromatin structure, thereby regulating the expression of various genes related to cell survival, growth, and differentiation, as well as genes regulating apoptosis [8]. Inhibition of HDACs is expected to induce cell cycle arrest and apoptosis in tumor cells, leading to an anticancer effect. HDACs are known to be overexpressed in many cancers, including BTC and PC [9, 10]. The active ingredient of S-1 is 5-fluorouracil (5-FU), and its active metabolite FdUMP forms a ternary complex with thymidylate synthase (TS; the rate-controlling enzyme for synthesis of pyrimidines required for DNA synthesis) and reduced folate, thereby inhibiting methylation of deoxyuridine monophosphate (dUMP) by TS to form thymidine monophosphate (dTMP) and thus interfering with DNA biosynthesis [11]. Much research has been undertaken on TS because this enzyme influences the sensitivity of tumor cells to 5-FU, and the findings have suggested that repeated administration of 5-FU may lead to overexpression of TS and tumor resistance [11, 12]. It was also reported that high TS gene expression predicted a poor response of metastatic tumors with shorter survival when patients were treated with S-1 alone [13].

In preclinical studies, resminostat downregulated TS expression and showed an additive antitumor effect when combined with S-1. Thus, in addition to its intrinsic antitumor activity, resminostat may overcome S-1 resistance by downregulation of TS.

Based on the above considerations, we conducted a phase I study in Japanese patients with pre-treated BTC or PC to assess the tolerability of combined therapy with resminostat/S-1 and to determine the optimal regimen for subsequent investigations.

Materials and methods

Patients

The main eligibility criteria were as follows: unresectable/recurrent BTC or unresectable/recurrent PC; at least one prior systemic chemotherapy regimen; Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1; age of 20–79 years; life expectancy ≥3 months; and adequate organ and bone marrow function (neutrophil count ≥1500/mm^3, platelet count ≥100,000/mm^3, serum total bilirubin ≤2.0 mg/dL, aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2.5 x the institutional upper limit of normal (ULN), serum creatinine ≤1.5 mg/dL, creatinine clearance ≥60 mL/min, and QTcF interval < 460 msec).

The main exclusion criteria were as follows: prior treatment with an HDAC inhibitor; prior treatment with S-1 (only for regimens 2 and 3); use of any other investigational drug within 20 days before enrollment; use of any other drug within 13 days before enrollment; cardiovascular complications or a history of myocardial infarction within 6 months before enrollment; symptomatic brain metastasis or suspected brain metastasis; and double cancer (synchronous double cancer or metachronous double cancer with a disease-free interval < 5 years).

This study was performed in accordance with the requirements of the Declaration of Helsinki and Good Clinical Practice, and was approved by the institutional review boards of all participating institutions. All patients provided written informed consent before enrollment. This study was registered with JAPIC Clinical Trials Information (JapicCTI-152,864).

Study design

An open-label phase I study was performed in two parts (Fig. 1). In part 1, the optimal regimen for resminostat/S-1 combination therapy was determined by using 3-patient cohorts, with up to 6 patients being enrolled sequentially to receive one of three regimens. Evaluation of dose-limiting toxicity (DLT) was the primary objective. In part 2, an additional 10 patients received the optimal resminostat/S-1 regimen determined in part 1 in order to confirm the tolerability and safety of this combination therapy. Secondary objectives were determination of the best overall response, PFS, overall survival (OS), and pharmacokinetics. As an exploratory analysis, expression of certain target genes in the peripheral blood was investigated to identify biomarkers that could predict the effect of resminostat/S-1 therapy.

Treatment

Three regimens were investigated in part 1 of the study. Regimen 1 was resminostat at 200 mg/day on Days 1 to 14. Regimen 2 was resminostat (200 mg/day) + S-1 (80–120 mg/

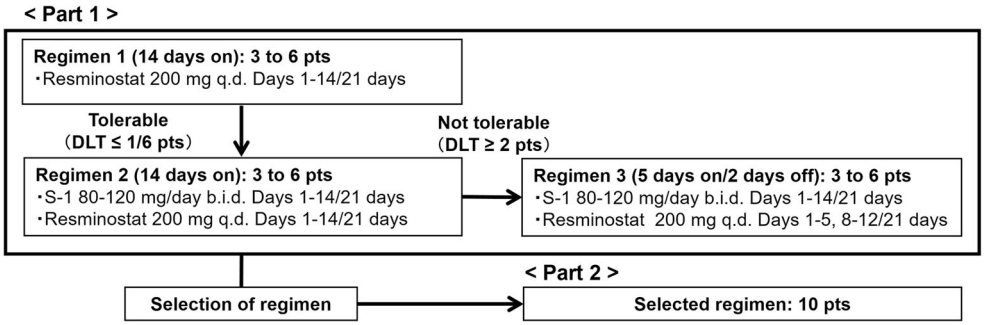

Fig. 1 Study design

day according to body surface area [BSA]) on Days 1 to 14. Regimen 3 was resminostat (200 mg/day on Days 1 to 5 and Days 8 to 12: 5 days on/2 days off) + S-1 (80–120 mg/day according to BSA on Days 1 to 14). Each regimen was repeated every 21 days.

DLT was defined as any of the following events during the first cycle of therapy in part 1 of the study: grade 4 thrombocytopenia; grade 4 neutropenia persisting for ≥8 days; febrile neutropenia with the temperature > 38.3°C at least once or ≥ 38.0°C for longer than 1 h; and other clinically relevant grade ≥ 3 non-hematologic toxicities. In addition, treatment-related adverse events that were considered to make continuation of treatment difficult were classified as DLTs after consultation with the Efficacy and Safety Assessment Committee. Three patients received each regimen. If DLT was observed in 0–1 of the 3 patients, an additional 3 patients were enrolled to receive the same regimen. If the tolerability of regimen 1 was confirmed, regimen 2 was tested subsequently. If regimen 2 was found to be intolerable, regimen 3 was tested. The regimen with which 2 or more patients experienced DLT was judged to be intolerable. The optimal regimen was determined by assessing treatment-related adverse events deemed to be DLTs that occurred during part 1.

Treatment with any of the 3 regimens was continued until progressive disease (PD) was confirmed, an adverse event leading to study discontinuation occurred, or another criterion for discontinuation was met such as withdrawal of consent. Dose modification was permitted for grade 4 neutropenia or thrombocytopenia, grade 3 QT interval prolongation, stomatitis, or diarrhea, and other clinically significant adverse events of grade 3. Increasing the dose of resminostat or S-1 was not allowed.

Assessment

Measurement of vital signs, laboratory tests, and recording the12-lead electrocardiogram were performed on Days 1, 8, and 15 of the first cycle and on Day 1 of the second and subsequent cycles. Adverse events were assessed according to Common Terminology Criteria for Adverse Events (version 4.03). Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (version 1.1) every 6 weeks from the date of first administration, and every 8 weeks from 24 weeks after the date of enrollment until PD. An outcome survey to assess OS was done at 6 months after the start of treatment in the last patient and was repeated every 3 months thereafter. The response rate (RR) was defined as the percentage of patients who had a best response rating of complete response or partial response according to RECIST. The diseases control rate (DCR) was defined as the percentage of patients who had a best response rating of complete response, partial response, or stable disease according to RECIST.

Pharmacokinetics

All patients enrolled in part 1 underwent measurement of the plasma concentrations of resminostat, tegafur, 5-FU, gimeracil, and oteracil potassium. Blood samples were collected before administration and at 1, 1.5, 2, 3, 4, 5, 6, 8 and 24 h after administration on Day 1 and Day 14 (Day 12) of the first cycle. Plasma drug concentrations were measured by liquid chromatography-tandem mass spectrometry. Analysis of pharmacokinetics was carried out using Phoenix WinNonlin, version 6.1 or 6.4 (Certana, St. Louis, MO, USA).

Biomarkers

In all patients enrolled in part 1, assessment of gene expression was performed before administration and at 1, 2, 5, and 24 h after administration on Day 1 and Day 14 (Day 12) of the first cycle. In all patients enrolled in part 2, gene expression was only assessed before treatment. Blood samples were collected from the patients by using a PAXgene Blood RNA kit (Qiagen, Hilden, Germany).

Expression of 11 genes (CCDC43, DEPDC7, DPP3, EXTL2, GTF3C6, HIST2H4, KDELC2, MICAL-L1, OAS2, ZFP64, and BIM) was determined because these genes have been suggested to be possible predictors of the therapeutic effect of resminostat by in vitro and clinical studies [14–17]. In addition, the expression of 4 genes (TS, DPD, OPRT, and TP) associated with sensitivity to 5-FU was measured [18, 19].

Gene expression in plasma samples was measured by the quantitative real-time reverse transcription polymerase chain reaction [20].

Statistical analysis

The 3-patient cohort method was employed in part 1 of this study. In part 2, enrollment of an additional 10 patients was considered necessary for careful assessment of the tolerability and safety of the regimen selected in part 1.

PFS and OS were evaluated by the Kaplan-Meier method and the 95% confidence intervals (CIs) were determined. The RR and DCR were calculated with 95% CIs based on the F distribution. In patients with a complete response or partial response (PR), the median time to response and the median overall response time, as well as the 95% CIs, were calculated by the Kaplan-Meier method.

PFS was defined as the period from Day 1 of the first cycle to the earliest event among PD or death from any cause. OS was defined as the period from Day 1 of the first cycle to the time of death from any cause.

The data cut-off date for analysis of efficacy and safety data was March 31, 2017 (after completion of 4 cycles by the last patient). However, OS was assessed from the outcome survey performed at 12 months after the start of treatment in the last patient.

To assess the potential usefulness of biomarkers for predicting the effect of resminostat/S-1 therapy, Cox regression was performed with expression levels of the target genes as covariates.

Statistical analysis was carried out using SAS version 9.3 or 9.4 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Between June 2015 and January 2017, a total of 27 patients were enrolled in the study at 2 institutions (part 1: regimen 1, 6 patients; regimen 2, 5 patients; regimen 3, 6 patients; part 2: 10 patients). All 27 patients received the study treatment and were evaluable for safety. Characteristics of the patients at enrollment are summarized in Table 1. Characteristics of the patients

Table 1 Characteristics of the patients

	Regimen 1 ($N = 6$)		Regimen 2 ($N = 5$)		Regimen 3 (N=16[a])	
	N	%	N	%	N	%
Age (years)						
Median	66.5		67.0		65.0	
Range	62–73		44–73		52–75	
Sex						
Male	3	50	4	80	10	63
Female	3	50	1	20	6	38
ECOG PS						
0	5	83	3	60	12	75
1	1	17	2	40	4	25
Primary tumor site						
IHBD	0	0	2	40	7	44
EHBD	2	33	1	20	1	6
GB	2	33	0	0	3	19
AV	0	0	0	0	2	13
Pancreas	2	33	2	40	3	19
Disease status						
Metastasis	4	67	3	60	9	56
Recurrence	2	33	2	40	7	44
Number of prior treatments						
1	2	33	4	80	13	81
≥2	4	67	1	20	3	19
Biliary drainage						
No	3	50	3	60	12	75
Yes	3	50	2	40	4	25

AV, ampulla of Vater; *ECOG*, Eastern Cooperative Oncology Group; *EHBD*, extrahepatic bile duct; *GB*, gallbladder; *IHBD*, intrahepatic bile duct; *PS*, performance status

[a] Total for study parts 1 and 2

who received regimen 3 ($n = 16$) were as follows. Their median age at the start of administration was 65.0 years (range: 52–75 years), and 12 patients (75%) had a PS of 0. Thirteen patients (81%) had BTC and 3 patients (19%) had PC. The number of previous chemotherapy regimens was 1 in 13 patients (81%; 12 patients with BTC and 1 patient with PC) and 2 or more in 3 patients (19%; 1 patient with BTC and 2 patients with PC). All 13 patients with BTC had received any of first-line treatments with a regimen that included GEM and cisplatin.

Safety

No DLT was observed in the 6 patients receiving regimen 1 in part 1 of the study, while DLT occurred in 1/5 patients receiving regimen 2 and 1/6 patients receiving regimen 3. Adverse events classified as DLTs were anorexia (grade 2) for regimen 2 and stomatitis (grade 3) for regimen 3. Although anorexia that occurred with regimen 2 was only grade 2, the event interfered with study drug treatment. Accordingly, the Efficacy and Safety Assessment Committee considered that it was significant with regard to assessment of tolerability and judged it to be a DLT. Among the 5 patients receiving regimen 2, only one DLT was noted, as described above, but adverse events requiring dose reduction also occurred in 4 patients during the second or third cycle. Thus, the Efficacy and Safety Assessment Committee concluded that regimen 2 was less tolerable, and decided that it was reasonable to stop further enrollment fir treatment with regimen 2 after 5 patients had been enrolled and proceed to regimen 3. Tolerability of regimen 3 was confirmed, with a DLT (stomatitis) occurring in 1/6 patients. Therefore, regimen 3 was considered to be the optimal regimen for part 2 of the study.

Treatment-related adverse events reported in ≥25% of all patients are shown in Table 2. All 27 patients treated during parts 1 and 2 of the study experienced at least one treatment-related adverse event. The most frequent treatment-related adverse events noted with regimen 3 were thrombocytopenia and anorexia (11 patients each, 69%), as well as neutropenia and nausea (7 patients each, 44%). The most common adverse events requiring delay of regimen 3 were thrombocytopenia (3 patients, 19%) and neutropenia (3 patients, 19%). The most common adverse event requiring suspension of regimen 3 was thrombocytopenia (2 patients, 13%). Serious treatment-related adverse events were not observed with regimen 1, but occurred in 1 patient (20%) receiving regimen 2 and 4 patients (25%) receiving regimen 3. There were no treatment-related deaths with any of the regimens.

Pharmacokinetics

Pharmacokinetics parameters of resminostat and 5-FU obtained with each regimen are shown in Table 3. No clear differences in the pharmacokinetic parameters of resminostat and 5-FU were noted among regimens 1, 2, and 3. In addition, the AUC and Cmax of resminostat and 5-FU with each regimen were similar to those reported with resminostat or S-1 monotherapy [21, 22]. Therefore, there was no evidence of interaction between resminostat and S-1.

Efficacy

Sixteen patients were treated with regimen 3 in parts 1 and 2, and the RR was 6.3% (1/16 patients, 95% CI: 0.2–30.2), being 7.7% (1/13 patients, 95% CI: 0.2–36.0)

Table 2 Treatment-related adverse events occurring in ≥25% of the patients receiving each regimen

	Regimen 1 (N = 6)		Regimen 2 (N = 5)		Regimen 3 (N = 16)	
	All Gr.	≥ Gr. 3	All Gr.	≥ Gr. 3	All Gr.	≥ Gr. 3
Adverse drug reactions, N (%)	6 (100)	0	5 (100)	1 (20)	16 (100)	7 (44)
Thrombocytopenia	3 (50)	0	4 (80)	0	11 (69)	3 (19)
Neutropenia	0	0	1 (20)	0	7 (44)	5 (31)
Leukopenia	0	0	1 (20)	0	6 (38)	3 (19)
Lymphopenia	0	0	2 (40)	0	3 (19)	2 (13)
Anemia	0	0	2 (40)	1 (20)	3 (19)	1 (6)
Anorexia	4 (67)	0	5 (100)	0	11 (69)	0
Nausea	2 (33)	0	4 (80)	0	7 (44)	0
Stomatitis	0	0	2 (40)	0	5 (31)	1 (6)
Vomiting	2 (33)	0	1 (20)	0	5 (31)	0
Skin hyperpigmentation	0	0	0	0	5 (31)	0
Malaise	0	0	1 (20)	0	4 (25)	0
Fatigue	2 (33)	0	3 (60)	0	3 (19)	1 (6)
Dysgeusia	2 (33)	0	0	0	2 (13)	0

Table 3 Pharmacokinetic parameters of resminostat and 5-FU

	Cycle 1, day 1					
	Resminostat (geometric mean, 68% range)			5-FU (mean, SD)		
	Regimen 1 (N = 6)	Regimen 2 (N = 5)	Regimen 3 (N = 6)	Regimen 1 (N = 6)	Regimen 2 (N = 5)	Regimen 3 (N = 6)
C_{max} (ng/mL)	1190 (791–1780)	1480 (1110 – 1970)	2000 (1290 – 3100)	–	140.4 (56.3)	175.4 (76.9)
T_{max} (h)	3.94[a] (1.09)	1.53[a] (1.06)	1.93[a] (0.42)	–	3.6 (0.5)	3.5 (1.0)
AUC_{inf} (h·ng/mL)	5720 (4690 – 6970)	5230 (3520 – 7760)	6830 (5230 – 8910)	–	880.6[b] (294.5)	1038[b] (545)
$t_{1/2}$ (h)	3.06 (2.74–3.41)	3.32 (2.94–3.76)	3.21 (3.11–3.32)	–	6.8 (2.3)	6.7 (2.2)
	Cycle 1, day 14 (day 12)					
	Resminostat (geometric mean, 68% range)			5-FU (mean, SD)		
	Regimen 1 (N = 6)	Regimen 2 (*N* = 3)	Regimen 3 (N = 6)	Regimen 1 (N = 6)	Regimen 2 (N = 3)	Regimen 3 (N = 6)
C_{max} (ng/mL)	1440 (671–3070)	799 (363–1760)	1670 (1240 – 2250)	–	120.4 (30.7)	136.9 (16.4)
T_{max} (h)	1.92[a] (2.54)	3.95[a] (2.26)	1.73[a] (0.89)	–	3.7 (0.6)	4.2 (0.8)
AUC_{inf} (h·ng/mL)	6670 (4660 – 9550)	3790 (1030 – 14,000)	7570 (5540 – 10,300)	–	980.2[b] (204.9)	934.1[b] (206.7)
$t_{1/2}$ (h)	3.69 (3.00–4.53)	3.76 (2.93–4.84)	3.35 (3.00–3.75)	–	9.3 (2.0)	8.6 (3.5)

AUC_{inf}, area under the plasma concentration vs. time curve from time zero to infinity; C_{max}, peak plasma concentration; T_{max}, time to reach the peak plasma concentration; $t_{1/2}$, half-life

[a] T_{max} values are shown as the mean (SD). [b] $AUC_{0–12}$

in patients with BTC and 0% (0/3 patients) in patients with PC. In the patient who achieved PR, the duration of response was 448 days. The DCR was 81.3% (13/16 patients, 95% CI: 54.4–96.0), being 84.6% (11/13 patients, 95% CI: 54.6–98.1) for BTC and 66.7% (2/3 patients) for PC (Table 4). The median PFS was 3.1 months (95% CI: 2.0–7.1), while it was 5.5 months (95% CI: 2.0–7.6) in patients with BTC (Fig. 2), and 2.3 months in patients with PC (Table 4). The median OS was 8.8 months (95% CI: 4.8–15.0) in all patients, being 10.2 months (95% CI: 6.1–20.5) in BTC (Fig. 3) and 4.7 months in PC (Table 4).

A waterfall plot and spider plot of the patients with BTC who received regimen 3 are shown in Fig. S1 and S2, respectively.

Biomarkers

Assessment of the relationship between differences in gene expression (≥ median / < median) before administration on Day 1 of the first cycle and the therapeutic effect did not identify any clear predictors of the efficacy of treatment. TS mRNA expression decreased and BIM mRNA expression increased in comparison with

Table 4 Efficacy of regimen 3

		All (N = 16) N (%) [95% CI]	BTC (*N* = 13) N (%) [95% CI]	PC (N = 3) N (%)
Best overall response	CR	0	0	0
	PR	1 (6.3)	1 (7.7)	0
	SD	12 (75.0)	10 (76.9)	2 (66.7)
	PD	2 (12.5)	1 (7.7)	1 (33.3)
	NE	1 (6.3)	1 (7.7)	0
Response rate (CR + PR)		1 (6.3) [0.2–30.2]	1 (7.7) [0.2–36.0]	0
Disease control rate (CR + PR + SD)		13 (81.3) [54.4–96.0]	11 (84.6) [54.6–98.1]	2 (66.7)
Median PFS (months)		3.1 [2.0–7.1]	5.5 [2.0–7.6]	2.3
Median OS (months)		8.8 [4.8–15.0]	10.2 [6.1–20.5]	4.7

BTC, biliary tract cancer; *CR*, complete response; *NE*, not evaluable; *OS*, overall survival; *PC*, pancreatic cancer; *PD*, progressive disease; *PFS*, progression-free survival; *PR*, partial response; *SD*, stable disease

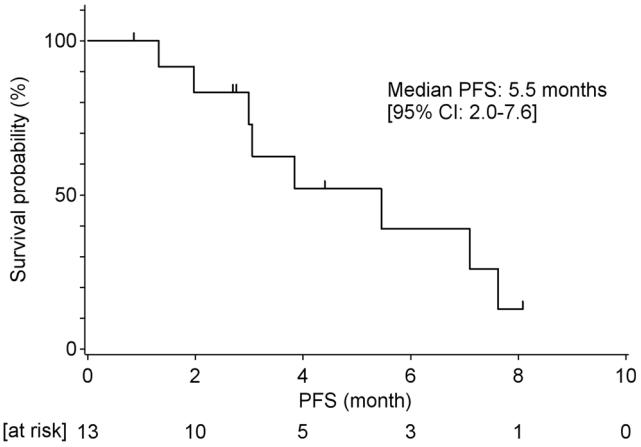

Fig. 2 Kaplan-Meier analysis of PFS in BTC patients who received regimen 3

pretreatment levels, while the other genes showed no variation.

Changes in the level of TS mRNA expression are shown in Table S1.

Discussion

In the present study, regimen 3 (resminostat/S-1 therapy with resminostat for 5 days on/2 days off) showed good tolerability in patients with pre-treated BTC or PC, suggesting that a phase II study of this regimen is warranted. The most frequent treatment-related adverse events caused by resminostat/S-1 therapy were thrombocytopenia, neutropenia, anorexia, and nausea. In particular, grade 3 or worse thrombocytopenia and neutropenia were observed more frequently than in previous studies of S-1 monotherapy [3, 4, 7], suggesting that concomitant use of resminostat and S-1 may increase the severity of these toxicities compared with S-1 monotherapy. However, all of the patients who developed grade 3 or worse thrombocytopenia or neutropenia recovered without any specific treatment. All cases of anorexia and nausea were grade 2 or less, and the incidence was comparable to that in previous studies of S-1 monotherapy [3, 4, 7]. In all patients, nausea could be controlled by antiemetic agents or dose modification. Therefore, adverse events related to resminostat/S-1 therapy were considered to be manageable.

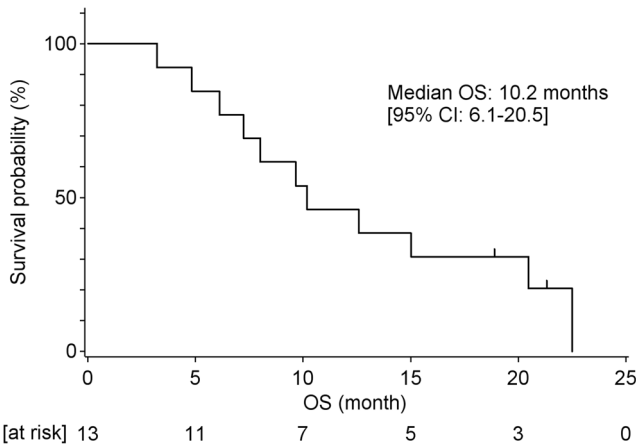

Fig. 3 Kaplan-Meier analysis of OS in BTC patients who received regimen 3

Prolongation of the QT interval on the electrocardiogram is considered to be a characteristic adverse event caused by HDAC inhibitors [23, 24]. In this study, 2 out of 27 patients treated with resminostat (1 receiving regimen 2 and 1 receiving regimen 3) showed grade 1 prolongation of the corrected QT interval. In both patients, the QT interval was normalized without any specific treatment and dose modification was not required for resminostat. In contrast to other studies of HDAC inhibitors [25–27], no prolongation of the corrected QT interval ≥ grade 2 was noted in this study.

With regard to efficacy, this study showed that the median PFS and OS for BTC patients were 5.5 months and 10.2 months, respectively. In addition, tumor shrinkage/stabilization was observed in all BTC patients who received the study treatment as second-line therapy, excluding one patient who received it as third-line therapy. Although our results were obtained in a small cohort of patients, they were favorable compared with those revealed by meta-analysis of previous studies investigating second-line therapy for BTC (PFS: 3.1–3.2 months, OS: 6.3–7.2 months, DCR: 50–62.5%) [28, 29]. In addition, regimen 3 was continued for ≥180 days in 3 patients, being continued for a long period of 448 days in the patient who achieved PR with this regimen. These results suggest that resminostat/S-1 therapy could be a promising second-line treatment for BTC.

Assessment of the efficacy of regimen 3 for PC was based on very limited data, since only 3 PC patients received this regimen (after 2 prior regimens in two patients and 1 in one patient). PFS and OS were 2.3 months and 4.7 months, respectively, which were comparable to the results of a previous study of second-line treatment for PC with S-1 (PFS was 2.0 months and OS was 4.5 months) [7]. Thus, further investigation of regimen 3 for PC is also warranted.

In this study, analysis of biomarker gene expression was performed on an exploratory basis to find mRNAs that could be used to predict the effect of resminostat/S-1 therapy. However, no such mRNAs were identified, possibly due to the small number of samples examined and the wide variation of gene expression.

Nonetheless, comparison of TS mRNA levels between before and after treatment revealed a decrease in TS expression in patients receiving resminostat/S-1 therapy. This result supports the hypothesis that suppression of TS expression (currently assumed to be the mechanism of action of resminostat) could enhance the antitumor effect of S-1, since TS overexpression may be associated with resistance to S-1.

In conclusion, regimen 3 (resminostat/S-1 therapy with resminostat for 5 days on/2 days off) was well tolerated and was selected as the recommended regimen for this combination therapy in pre-treated patients with BTC or PC. The efficacy and safety of resminostat/S-1 therapy need to be evaluated further in this patient population by additional prospective clinical studies. Currently, a multicenter randomized controlled phase II study is being planned in BTC patients pretreated with GEM plus a platinum agent.

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Compliance with ethical standards

Conflict of interest Masafumi Ikeda has received research grants from Bayer Yakuhin, Kyowa Hakko Kirin, Yakult Honsha, Taiho Pharmaceutical, Eli Lilly Japan, Ono Pharmaceutical, Eisai, AstraZeneca, Zeria Pharmaceutical, Baxalta Japan Limited, Chugai Pharmaceutical, Bristol-Myers Squibb, Merck Serono, Kowa, Nano Carrier, ASLAN Pharmaceuticals, Novartis Pharma, and Takara Bio. He has also received honoraria from Novartis Pharma, Bayer Yakuhin, Bristol-Myers Squibb, Abbott Japan, Eisai, Taiho Pharmaceutical, Eli Lilly Japan, Daiichi-Sankyo, Yakult Honsha, Otsuka Pharmaceutical, Nobelpharma, Sumitomo Dainippon Pharma, and Teijin Pharma. Furthermore, he has received consulting fees from Nano Carrier, Bayer Yakuhin, Eisai, Kyowa Hakko Kirin, Novartis Pharma, Shire, MSD, Bristol-Myers Squibb, Teijin Pharma, and Daiichi-Sankyo. Izumi Ohno has received a consulting fee from Merck Serono. Hideki Ueno has received research grants from Taiho, Eli Lilly, Nano Carrier, Baxalta, and Yakult Honsha, as well as honoraria from Taiho, Chugai, and Yakult Honsha. Shuichi Mitsunaga has received research grants from Bayer Yakuhin, Chugai Pharmaceutical, Merck Serono, ASLAN Pharmaceuticals, TORAY, Ono Pharmaceutical, and Sumitomo Dainippon Pharma, as well as honoraria from Ono Pharmaceutical and TORAY. Yusuke Hashimoto has received research grants from Taiho Pharmaceutical and Takara Bio Company. He has also received honoraria from Taiho Pharmaceutical, Boston Scientific, and Olympus Medical, as well as travel accommodation from Taiho Pharmaceutical and a consulting fee from Medicos Hirata. Takuji Okusaka has received research grants from Eli Lilly Japan, Eisai, Novartis Pharma, Yakult Honsha, Taiho Pharmaceutical, Nippon Boehringer Ingelheim, Kowa Company, Kyowa Hakko Kirin, Merck Serono, Ono Pharmaceutical, Bayer Yakuhin, Pfizer Japan, AstraZeneca, Dainippon Sumitomo Pharma, Nobelpharma, Zeria Pharmaceutical, Glaxo Smith Kline, Shizuoka Industry, Nano Carrier, Baxter, and Chugai Pharmaceutical. He has also received honoraria from Novartis Pharma, Taiho Pharmaceutical, Merck Serono, Eli Lilly Japan, Dainippon Sumitomo Pharma, Bayer Yakuhin, Yakult Honsha, Nobelpharma, Nippon Kayaku, Baxter, FUJIFILM RI Pharma, AstraZeneca, Ono Pharmaceutical, EA Pharma, Nippon Chemiphar, Celgene, and Chugai Pharmaceutical. Furthermore, he has received consulting fees from Eli Lilly Japan, Dainippon Sumitomo Pharma, Taiho Pharmaceutical, Nippon Boehringer Ingelheim, Zeria Pharmaceutical, and Daiichi Sankyo. Hideaki Takahashi has received research grants from Bayer Pharmaceutical and Bristol-Myers Squibb, as well as honoraria from Taiho Pharmaceutical and travel accommodation from Pfizer Inc. Rina Hara, Shingo Kobayashi, and Osamu Nakamura are employees of Yakult Honsha. Rina Hara also owns stock in Yakult Honsha. Chigusa Morizane has received research grants from GlaxoSmithKline, Pfizer, Nobelpharma, Eisai, Yakult Honsha, Ono Pharmaceutical, and Taiho Pharmaceutical. He has also received honoraria from Pfizer, Novartis, Yakult Honsha, Lilly, Nobelpharma, and Fujifilm. Furthermore, he has received consulting fees from AstraZeneca, Yakult Honsha, Novartis, and Taiho Pharmaceutical. No potential conflicts of interest were disclosed by the other authors.

Ethical approval This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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