Human equilibrative nucleoside transporter-1 expression is a predictor in patients with resected pancreatic cancer treated with adjuvant S-1 chemotherapy

Yukiyasu Okamura1 | Satoru Yasukawa2 | Hiroto Narimatsu3 | Narikazu Boku4 | Akira Fukutomi5 | Masaru Konishi6 | Soichiro Morinaga7 | Hirochika Toyama8 | Yuji Kaneoka9 | Yasuhiro Shimizu10 | Shoji Nakamori11 | Naohiro Sata12 | Keisuke Yamakita13 | Amane Takahashi14 | Osamu Kainuma15 | Shoichi Hishinuma16 | Ryuzo Yamaguchi17 | Masato Nagino18 | Satoshi Hirano19 | Akio Yanagisawa2 | Keita Mori20 | Katsuhiko Uesaka1

1Division of Hepato-Biliary-Pancreatic Surgery, Shizuoka Cancer Center Hospital, Nagaizumi, Japan
2Pathology, Kyoto Prefectural University of Medicine, Kyoto, Japan
3Cancer Prevention and Control Division, Kanagawa Cancer Center, Yokohama, Japan
4Gastrointestinal Medical Oncology, National Cancer Center Hospital, Tokyo, Japan
5Gastrointestinal Oncology, Shizuoka Cancer Center, Shizuoka, Japan
6Hepato-Biliary-Pancreatic Surgery, National Cancer Center Hospital East, Kashiwa, Japan
7Department of Gastrointestinal Surgery, Kanagawa Cancer Center, Yokohama, Japan
8Hepato-Biliary-Pancreatic Surgery, Kobe University, Kobe, Japan
9Surgery, Ogaki Municipal Hospital, Ogaki, Japan
10Gastrointestinal Surgery, Aichi Cancer Center Hospital, Nagoya, Japan
11Surgery, National Hospital Organization Osaka National Hospital, Osaka, Japan
12Gastrointestinal Surgery, Jichi Medical University, Shimotsuke, Japan
13Division of Metabolism and Biosystemic Science, Department of Medicine, Asahikawa Medical University, Asahikawa, Japan
14Gastrointestinal Surgery, Saitama Cancer Center, Saitama, Japan
15Gastrointestinal Surgery, Chiba Cancer Center, Chiba, Japan
16Surgery, Tochigi Cancer Center, Utsunomiya, Japan
17Surgery, Kasugai Municipal Hospital, Kasugai, Japan
18Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan
19Gastroenterological Surgery II, Faculty of Medicine, Hokkaido University, Sapporo, Japan
20Clinical Trial Coordination Office Biostatistician, Shizuoka Cancer Center Hospital, Nagaizumi, Japan

Correspondence
Yukiyasu Okamura, Division of Hepato-Biliary-Pancreatic Surgery, Shizuoka Cancer Center Hospital, 1007 Shimo-Nagakubo, Sunto-Nagaizumi, Shizuoka 411-8777 Japan.
Email: yu.okamura@scchr.jp

Abstract
The high expression of human equilibrative nucleoside transporter-1 (hENT1) and the low expression of dihydropyrimidine dehydrogenase (DPD) are reported to predict a favorable prognosis in patients treated with gemcitabine (GEM) and 5-fluorouracil (5FU) as the adjuvant setting, respectively. The expression of hENT1 and DPD were analyzed in patients registered in the JASPAC 01 trial, which showed a better...
Pancreatic cancer is one of the most aggressive and devastating malignant solid tumors, and the mortality rate is rising. Most patients have unresectable status with distant metastases, and surgical resection is possible in approximately 10% of all pancreatic cancer patients. Introducing adjuvant chemotherapy leads to more than a doubling of the 5-year survival rate, from approximately 10% with surgery alone to approximately 44%, in patients with resectable disease. Disease-free and overall survival rates could be improved by adjuvant chemotherapy with 5-fluorouracil (5-FU) and folinic acid (FA), or gemcitabine (GEM) monotherapy for 6 months following pancreatectomy.

Japan Adjuvant Study Group of Pancreatic Cancer (JASPAC) 01 was a randomized, controlled phase III trial. Comparing S-1 with GEM as adjuvant chemotherapy for patients with pancreatic cancer, the study confirmed the superiority of S-1 (TS-1; Taiho Pharmaceutical) to GEM. Long-term survival was obtained in some patients of the GEM group, while some patients had early recurrence in the S-1 group, despite the fact that the prognosis of the S-1 group, on the whole, was better than for the GEM group in the JASPAC 01 study. Although more targeted therapies may be possible with improved understanding of the molecular pathology of pancreatic cancer, there is the potential for improved outcomes based on current therapies using appropriate biomarkers. The JASPAC 01 study is an ideal tool for biomarker analyses to predict the efficacy of GEM and S-1 for pancreatic cancer because it provides not only prospectively collected data but also more than 5 years of follow-up data.

1 | INTRODUCTION

The human equilibrative nucleoside transporter 1 (hENT1), which controls the bidirectional passage into cells of pyrimidine nucleosides such as GEM, capecitabine and 5-FU, is a promising biomarker. Dihydropyrimidine dehydrogenase (DPD), which is a rate-limiting enzyme in 5-FU catabolism, is another candidate. The correlations between the expression levels of these biomarkers in tumor specimens and clinical outcomes have been shown. Many studies have suggested that their expression level could accurately predict the clinical outcome in patients receiving fluoropyrimidine-based chemotherapy or GEM. However, there is no consensus about the clinical importance of the expressions of these genes, as each study has different results, and most published reports concern relatively small randomized studies or retrospective analyses.

We assessed the expression of hENT1 and DPD genes by immunohistochemistry staining using specimens obtained from patients registered in the JASPAC 01 study. The main aim of the present study was to determine whether hENT1 and/or DPD expressions in tumor tissue would help predict the outcomes for the patients treated with S-1 or GEM.

2 | MATERIALS AND METHODS

2.1 | Study population and design

We retrospectively designed this biomarker study, after the completion of the final analysis of the JASPAC 01, to investigate whether hENT1 and/or DPD could predict a prognostic benefit of S-1 and/or GEM, and
collected the tumor tissue from patients registered in the JASPAC 01.5
Unstained slides made by formalin-fixed, paraffin-embedded (FFPE) surgical resection specimens stocked at each of the 24 collaborating institution were collected for 326 of all 377 (86.5%) patients enrolled in the JASPAC 01 and the biomarker study population comprised 326 patients (Figure 1). The protocol of this biomarker study was approved by the ethics committee of the Shizuoka Cancer Center "27-22-27-1-5" and each collaborating institutional review board.

2.2 | Immunohistochemistry

Immunohistochemistry (IHC) for hENT1 was carried out on the unstained slides (thickness: 3-5-μm) according to the standard protocol (anti-hENT1 rabbit monoclonal antibody SP120, Roche Tissue Diagnostics; already diluted antibody) and IHC for DPD was also performed (anti–DPD mouse monoclonal antibody ADPYDMAB, Immune-Biological Laboratories; 1:50). These antibodies were tested for specific binding in vitro using western blotting.

Two independent pathologists (SY and AY), who were blinded to all clinical information, evaluated the intensity of staining using light microscopy. The intensity of the DPD and hENT1 expression was evaluated as previously reported.16,20 Pancreatic islet cells were used as an internal positive control for anti–DPD and anti–hENT1 staining because DPD and hENT1 are strongly expressed in islet cells.15 The intensity of tumor cell immunostaining was classified into four groups as follows: no, not stained; weak, <50% of the tumor cells were weakly stained in comparison to positive control; moderate, <50% of the tumor cells were strongly stained in comparison to positive control or weakly stained compared to positive control in 50% or ≥ 50% of the tumor cells were weakly stained in comparison to positive control; strong, ≥50% of the tumor cells were strongly stained in comparison to positive control. The high and low expression of hENT1 and DPD was defined as strong/moderate staining and no/weak staining, respectively. Ambiguous cases were discussed to obtain agreement each other.

2.3 | Baseline data

JASPAC 01 was a randomized phase 3 trial comparing adjuvant S-1 versus adjuvant GEM in patients who underwent curative resection for pancreatic cancer.5 One cycle treatment with GEM (1000 mg/m² on days 1, 8 and 15) followed by a 1-week rest period was repeated every 4 weeks for 24 weeks in an outpatient setting, and patients assigned to the S-1 group received S-1 (40 mg for a body surface area less than 1.25 m², 50 mg for a body surface area of 1.25 m² to 1.5 m², or 60 mg for a body surface area ≥ 1.5 m²), twice oral intake per day for 28 consecutive days followed by a 14-day rest (one cycle). This administration of S-1 was repeated every 6 weeks for up to four cycles.5 In both groups, subsequent follow-up examinations were performed at 3-month intervals. In this study, a total of 377 patients who underwent macroscopic curative resection for pancreatic cancer (R0 and R1 resection) were finally recruited between April 2007 and June 2010.

2.4 | Statistical analysis

The χ²-test or Fisher’s exact test was used for comparisons between the categorical variables. Continuous variables are compared using the Mann-Whitney U-test and are shown as the median and range. The survival rate was calculated using the Kaplan-Meier method and compared using the log-rank test. A Cox proportional hazards model was used for the univariate and multivariate analyses, and all factors found to be significant predictors (P < .05) in the univariate analysis were entered into the multivariate analysis. The multivariate analysis was performed according to the logistic regression method using a backward stepwise selection model. All statistical analyses were performed using the SPSS 26.0 software package (SPSS). Two-tailed P-values of < 0.05 were considered to indicate statistical significance.

We estimated the minimum difference in survival that would be required to show a significant difference in survival between patients with tumors in which gene expression was high or low in each treatment arm. Given a tertile or median cutoff point, demonstrating a statistically significant difference in survival between patients with tumors with high and low gene expression levels would require hazard ratios of at least 0.51 and 0.57, respectively, assuming a two-sided α value of 0.05 and a power of 80% in a proportional hazards model. Thus, we determined that each arm should include approximately 150 patients.

3 | RESULTS

3.1 | Patient characteristics

No statistical difference was identified between the population used in the present study and the total population of the JAPAC 01 study (Table 1).3 The gene expression levels and other factors in enrolled patients were well balanced between the S-1-treated and GEM-treated groups.
3.2 | Expression of human equilibrative nucleoside transporter-1 and dihydropyrimidine dehydrogenase according to immunohistochemistry

The strong, moderate and weak immunohistochemical expressions of hENT1 in the tumor were identified in 31, 69 and 146 patients, respectively; no expression was detected in 80 patients (Figure 2A-D). The strong, moderate and weak immunohistochemical expressions of DPD in the tumor were identified in 9, 54 and 168 patients, respectively; no expression was detected in 95 patients (Figure 3A-D). As a result, the high expression of hENT1 and DPD was identified in 100 and 63 of 319 patients (30.7% and 19.3%), respectively.

| TABLE 1 Baseline characteristics of the patients in the study |
|---------------------------------------------------------------|
| **Characteristics** | **Overall** | **hENT1 expression** | **DPD expression** |
| | N = 326 | High (N = 100 (30.7%)) | Low (N = 226 (69.3%)) | P | High (N = 63 (19.3%)) | Low (N = 263 (80.7%)) | P |
| Sex (number, %) | | | | | | | |
| Male | 181 (55.5) | 58 (58.0) | 123 (54.4) | .549 | 38 (60.3) | 143 (64.4) | .349 |
| Female | 145 (44.5) | 42 (42.0) | 103 (45.6) | 25 (39.7) | 120 (45.6) | | |
| Age<sup>a</sup> | 66 (34-86) | 65 (44-82) | 67 (34-86) | .218 | 66 (37-83) | 66 (34-86) | .432 |
| ECOG performance status (number, %) | | | | | | | |
| 0 | 220 (67.5) | 62 (62.0) | 158 (69.9) | .160 | 32 (50.8) | 188 (71.5) | .002 |
| 1 | 106 (32.5) | 38 (38.0) | 68 (30.1) | | 31 (49.2) | 75 (28.5) | |
| Survival time (years) | | | | | | | |
| Median RFS (95% CI) | 1.29 (1.08-1.54) | 1.18 (0.97-1.39) | 1.51 (1.08-1.94) | .092 | 1.27 (0.80-1.73) | 1.43 (1.08-1.78) | .702 |
| Median OS (95% CI) | 2.85 (2.46-3.49) | 2.43 (1.59-3.28) | 3.42 (2.67-4.17) | .091 | 3.31 (1.64-4.99) | 2.90 (2.30-3.50) | .525 |
| Treatment arm (number, %) | | | | | | | |
| Gemcitabine | 166 (50.9) | 51 (51.0) | 115 (50.9) | .985 | 31 (49.2) | 135 (51.3) | .762 |
| S-1 | 160 (49.1) | 49 (49.0) | 111 (49.1) | 32 (50.8) | 128 (48.7) | | |
| Residual tumor status (number, %) | | | | | | | |
| R0 | 283 (86.8) | 87 (87.0) | 196 (86.7) | .946 | 54 (85.7) | 229 (87.1) | .075 |
| R1 | 43 (13.2) | 13 (13.0) | 30 (13.3) | 9 (14.3) | 34 (12.9) | | |
| Primary tumor status (number, %)<sup>b</sup> | | | | | | | |
| T1-T2 | 36 (11.0) | 10 (10.0) | 26 (11.5) | .689 | 5 (7.6) | 31 (11.8) | .381 |
| T3-T4 | 290 (89.0) | 90 (90.0) | 200 (88.5) | 58 (92.4) | 232 (88.2) | | |
| Regional lymph node status (number, %)<sup>b</sup> | | | | | | | |
| N0 | 114 (35.0) | 28 (28.0) | 86 (38.1) | .079 | 23 (36.5) | 91 (34.6) | .776 |
| N1 | 212 (65.0) | 72 (72.0) | 140 (61.9) | 40 (63.5) | 172 (65.4) | | |
| CA19-9 (median, IQR) | | | | | | | |
| ≤37 U/mL | 257 (78.8) | 75 (75.0) | 182 (80.5) | .330 | 48 (76.2) | 209 (79.5) | .721 |
| >37 U/mL | 68 (20.9) | 24 (24.0) | 44 (19.5) | 14 (22.2) | 54 (20.5) | | |
| Pathological stage (number, %)<sup>b</sup> | | | | | | | |
| IA | 17 (75.0) | 2 (2.0) | 15 (6.6) | .203 | 2 (3.2) | 15 (5.7) | .803 |
| IB | 8 (24.0) | 3 (3.0) | 5 (2.2) | 1 (1.6) | 7 (2.7) | | |
| IIA | 88 (75.0) | 23 (23.0) | 65 (28.8) | 19 (30.2) | 69 (26.2) | | |
| IIB | 211 (24.0) | 72 (72.0) | 139 (61.5) | 41 (65.1) | 170 (64.6) | | |
| III | 2 (75.0) | 0 | 2 (0.9) | 0 | 2 (0.8) | | |

Note: The values in parentheses are percentages unless indicated otherwise.

DPD, dihydropyrimidine dehydrogenase; ECOG, Eastern Cooperative Oncology Group; hENT1, human equilibrative nucleoside transporter-1; IQR, interquartile range.

<sup>a</sup>Value is median (range).

<sup>b</sup>Primary tumor status, regional lymph node status and pathological stage were described according to the TNM Classification of Malignant Tumours, 6th edition.
3.3 | Correlation between the cytoplasmic human equilibrative nucleoside transporter-1 and dihydropyrimidine dehydrogenase expression and survival

The median overall survival was 25.9 months in the GEM-treated patients (95% confidence interval [CI] 20.6-31.2), while the median overall survival was 44.6 months (95% CI 31.5-57.7) in the S-1-treated patients (hazard ratio [HR] 0.62, 95% CI 0.47-0.80, \( P < 0.001 \)).

The median relapse-free survival of the GEM-treated patients was 13.4 months (95% CI 10.4-16.4), while that of the S-1-treated patients was 22.6 months (95% CI 31.5-57.7; HR 0.68, 95% CI 0.52-0.87, \( P = 0.003 \)).

According to the hENT1 expression (Figure 4), the median overall survival in the GEM-treated patients with low hENT1 was 26.1 months (95% CI 19.7-32.5), while that with high hENT1 patients was 25.5 months (95% CI 19.3-31.7). The HR was 1.05 (95% CI 0.72-1.53, \( P = .786 \)), with no significant difference (Figure 5A). Unexpectedly, the median overall survival in the S-1-treated patients with low hENT1 was 58.0 months (95% CI 30.9-85.0), which was significantly better than that with high hENT1 (30.9 months: 95% CI 21.4-40.4). The HR was 1.75 (95% CI 1.16-2.64, \( P = 0.007 \)) for high hENT1 to low hENT1 (Figure 5B).

The median relapse-free survival of the GEM-treated patients with low hENT1 was 12.6 months (95% CI 8.5-16.7), while that of the patients with high hENT1 was 13.5 months (95% CI 8.1-19.0). The HR was 1.01 (95% CI 0.69-1.46, \( P = .979 \)). The median relapse-free survival of the S-1-treated patients with low hENT1 was 28.4 months (95% CI 19.2-37.6), which was significantly better than that of the patients with high hENT1 (15.1 months: 95% CI 8.2-21.9). The HR for high hENT1 to low hENT1 was 1.60 (95% CI 1.07-2.38, \( P = 0.022 \)).

According to the DPD expressions (Figure 6A), the median overall survival of the GEM-treated patients with low DPD was 26.1 months (95% CI 21.9-30.3) while that of the high DPD patients was 25.5 months (95% CI 0.4-50.6). The HR was 0.85 (95% CI 0.56-1.30, \( P = .445 \)) (Figure 6B). The median overall survival in the S-1-treated patients with low DPD was 44.6 months (95% CI 31.6-57.7) while...
that of the high DPD patients was 42.7 months (95% CI 0.4-50.6). The HR was 1.05 (95% CI 0.65-1.72, \( P = 0.833 \)) (Figure 6C).

The median relapse-free survival of the GEM-treated patients with low DPD was 12.9 months (95% CI 8.8-16.9), while that of the patients with high DPD was 14.7 months (95% CI 9.35-20.0). The HR was 0.94 (95% CI 0.61-1.45, \( P = 0.794 \)). The median relapse-free survival of the S-1-treated patients with low DPD was 23.2 months (95% CI 14.3-32.1), while that the patients with high DPD was 17.9 months (95% CI 5.9-29.9). The HR was 1.08 (95% CI 0.68-1.71, \( P = 0.744 \)).

### 3.4 The forest plot analysis for overall survival

The forest plot analysis revealed that HR were higher than 1.0 in the subgroups of patients with strong expression of hENT1 and histological types other than tubular adenocarcinoma. However, there were small numbers of patients in these subgroups (Figure 7). Although we defined the high and low expression of hENT1 and DPD as strong/moderate staining and no/weak staining in the current study, there was no cutoff point of IHC staining that could identify the patients who should receive GEM therapy rather than S-1 therapy based on the hENT1 and DPD expression.

### 3.5 Predictors for overall survival in S-1-treated patients

The multivariate analysis showed that CA19-9 level > 37 U/mL, lymph node metastasis positivity, residual tumor status R1, and a moderate or strong hENT1 expression on IHC were significant predictors for overall survival (Table 2). The multivariate analysis showed that lymph node metastasis, residual tumor status R1 and positive staining (intensity, moderate or strong) of hENT1 on IHC were significant predictors of relapse-free survival (Table 2).

### 3.6 Predictors of overall and relapse-free survival in gemcitabine-treated patients

The multivariate analysis showed that T factor (T3-4) was a significant predictor of overall and relapse-free survival and the regional lymph node status being positive was a significant predictor of relapse-free survival (Table 3).

### 4 DISCUSSION

The present study was performed based on the hypothesis that hENT is a useful biomarker for efficacy of GEM and that DPD is a useful biomarker for S-1. However, we failed to prove the hypothesis and unexpectedly found that hENT1 is a candidate biomarker for S-1.

The JASPAC 01 study showed that the HR for mortality in S-1-treated patients, in comparison to GEM-treated patients, was 0.57 (the estimated 5-year overall survival rate in the S-1 group was 44.1%, while that in the GEM group was 24.4%); however, there may be some patients for whom GEM is more effective than S-1. From the previous paper’s results,\textsuperscript{11-18} the high expression of hENT1
is considered to be a favorable predictor in GEM-treated patients and a low DPD expression is considered to be a favorable predictor for S-1-treated patients. Thus, it can be hypothesized that patients with high hENT1 and DPD expression levels may have high response to GEM rather than S-1. If we could select such patients before adjuvant therapy, the 5-year overall survival rate after surgery for pancreatic cancer might reach over 50%. The final goal of the present study was to establish the appropriate use of individualized adjuvant chemotherapy with S-1 or GEM.

Unexpectedly, the present study showed that hENT1 is a candidate biomarker for S-1. Although the hENT1 expression has been known as a biomarker of GEM, researchers have paid little attention to the relationship between hENT1 expression and other anti-cancer drugs. Tsujie et al showed that hENT1 mRNA levels might predict the 5-FU sensitivity for pancreatic cancer. Although the results of the present study used immunohistochemistry rather than mRNA levels, they were consistent with the result of Tsujie et al. However, its mechanism has not been clarified, which should be investigated in the future. Because the present study showed no advantage, in terms of survival, in patients with high hENT1 expression levels who were treated with S-1 (in comparison to GEM), GEM may also be the first choice for such patients. In contrast, the 5-year overall survival rate in the S-1-treated patients with low hENT1 exceeded 50% and S-1 was the best treatment for such patients.

Regarding hENT1, some studies have shown that hENT1 can predict the treatment outcomes in patients treated with GEM but not

**Figures**: A. Kaplan-Meier survival curves for overall survival in the gemcitabine arm after the stratification by human equilibrative nucleoside transporter-1 (hENT1) expression. B. Kaplan-Meier survival curves for overall survival in the S-1 arm after the stratification by human equilibrative nucleoside transporter-1 (hENT1) expression.
those treated with S-1 for pancreatic ductal adenocarcinoma.\textsuperscript{12-15} Notably, all studies were performed using the mouse monoclonal antibody 10D7G2, which is not commercially available. Moreover, the scoring systems to assign patients to high or low hENT1 expression subgroups were different among these investigations, despite using the same antibody. Several retrospective studies with small cohorts have shown that the hENT1 expression, which was assessed using a rabbit polyclonal antibody, is useful for predicting the survival of pancreatic cancer patients who were treated with GEM.\textsuperscript{16-18} In contrast, Sinn et al showed that the hENT1 expression using the monoclonal rabbit antibody SP120 does not predict the survival of pancreatic cancer patients who receive GEM as adjuvant treatment.\textsuperscript{19} The results of the present study were consistent with Sinn’s results and were in contrast to those of the abovementioned studies.

In addition to Sinn’s report, which was based on the CONKO-001 study,\textsuperscript{20} the results of the present study are in line with those of other studies using SP120 for the evaluation of hENT1 expression.\textsuperscript{21,22} In the GEM-treated patients of both the AIO-PK study\textsuperscript{21} and the CO-101 study,\textsuperscript{22} which used the SP120 antibody, no relationship was demonstrated between the expression of hENT1 and survival. Although the review article related to this issue concluded that the expression of hENT1 is an appropriate biomarker for predicting the outcomes of patients undergoing adjuvant GEM-based chemotherapy,\textsuperscript{24} the hENT1 expression level has no clinical utility in any studies in which SP120 was used to evaluate the expression of hENT1. The present study is the fifth study using specimens from a prospective, randomized controlled study. A reproducible standard procedure in which the antibody and cutoff points are standardized is urgently needed before the implementation of hENT1 as a predictive biomarker in pancreatic cancer treatment. If 10D7G2 is a promising antibody for detecting the expression of hENT1, surgeons and physicians who treat pancreatic cancer would want it to be commercially obtained.

S-1, an oral 5-FU prodrug, which has been developed in Japan, consists of tegafu (a prodrug of 5-FU), gimeracil (a potent DPD inhibitor) and oteracil (an inhibitor of 5-FU phosphorylation in the gastrointestinal tract) in a 1:0.4:1 molar concentration ratio.\textsuperscript{25} As mentioned above, some papers have shown that DPD is a useful biomarker for S-1.\textsuperscript{16} However, S-1 is used in East Asia, mainly in Japan, and is not widely used elsewhere in the world due to its side effects. Thus, most published reports have involved relatively small randomized or retrospective analyses from nonrandomized studies, and no report has shown the utility of the hENT1 and DPD expression for...
**FIGURE 7** Forest plot analyses for overall survival. AC, adenocarcinoma; DP, distal pancreatectomy; HR, hazard ratio; PD, pancreatoduodenectomy; PS, Eastern Cooperative Oncology Group performance status; R0, no residual tumor; R1, microscopic presence of tumor cells at the surface of the resection margin; T stage and N stage, TNM classification of malignant tumors.
| Variables                                      | Overall survival | Relapse-free survival |
|-----------------------------------------------|------------------|-----------------------|
|                                               | Univariate       | Multivariate          | Univariate | Multivariate |
|                                               | Hazard ratio (95% confidence interval) | P | Hazard ratio (95% confidence interval) | P | Hazard ratio (95% confidence interval) | P |
| Sex (male/female)                             | 1.07 (0.72-1.61) | .728                  | 0.98 (0.67-1.44) | .932 |
| Age (≥65 years/<65 years)                     | 1.19 (0.79-1.79) | .404                  | 1.15 (0.78-1.70) | .478 |
| ECOG performance status (1/0)                 | 1.08 (0.70-1.67) | .716                  | 1.02 (0.67-1.54) | .939 |
| Residual tumor status (R1/R0)                 | 1.89 (1.10-3.23) | .020                  | 1.90 (1.13-3.21) | .016 |
| Primary tumor status (T3-T4/T1-2)             | 1.50 (0.73-3.10) | .269                  | 1.49 (0.75-2.94) | .257 |
| Regional lymph node status (N1/N0)            | 2.02 (1.26-3.25) | .004                  | 1.92 (1.23-2.99) | .004 |
| CA19-9 (>37 U/mL/≤37 U/mL)                    | 2.11 (1.35-3.29) | .001                  | 1.94 (1.25-3.03) | .003 |
| hENT1 expressions of IHC                      | Weak or more/No  | 1.65 (0.98-2.78)      | 1.83 (1.10-3.05) | .019 |
|                                               | Moderate or strong (positive)/No or weak (negative) | 1.75 (1.16-2.64) | .008 | 1.61 (1.06-2.45) | .027 |
|                                               | Strong/Moderate or less | 1.51 (0.81-2.83) | .200 | 1.67 (0.93-2.98) | .084 |
| DPD expressions of IHC                        | Weak or more/No  | 1.13 (0.71-1.83)      | 1.04 (0.67-1.61) | .852 |
|                                               | Moderate or strong (positive)/No or weak (negative) | 1.05 (0.65-1.72) | .833 | 1.08 (0.68-1.71) | .744 |
|                                               | Strong/Moderate or less | 0.92 (0.23-3.73) | .905 | 0.76 (0.19-3.06) | .694 |

DPD, dihydropyrimidine dehydrogenase; ECOG, Eastern Cooperative Oncology Group; hENT1, human equilibrative nucleoside transporter-1; IHC, immunohistochemistry.
| Variables | Overall survival | | | Relapse-free survival | | |
|---|---|---|---|---|---|---|
| | Univariate | Multivariate | Univariate | Multivariate |
| | Hazard ratio (95% confidence interval) | P | | Hazard ratio (95% confidence interval) | P | |
| Sex (male/female) | 1.24 (0.88-1.75) | .219 | | | |
| Age (≥65 years/<65 years) | 1.00 (0.98-1.02) | .404 | | 0.91 (0.64-1.30) | .912 | |
| ECOG performance status (1/0) | 1.46 (1.02-2.09) | .037 | | 1.41 (0.98-2.02) | .066 | |
| Residual tumor status (R1/R0) | 1.99 (1.24-3.32) | .004 | | 1.73 (1.07-2.81) | .025 | |
| Primary tumor status (T3-T4/ T1-2) | 4.22 (1.97-9.07) | <.001 | 3.39 (1.55-7.39) | .002 | 3.95 (1.93-8.11) | <.001 | 3.26 (1.57-6.77) | .002 |
| Regional lymph node status (N1/ N0) | 1.89 (1.30-2.75) | .001 | | 1.98 (1.36-2.88) | <.001 | 1.59 (1.08-2.34) | .019 | |
| CA19-9 (>37 U/mL/≤37 U/mL) | 1.84 (1.24-2.73) | .003 | | 1.76 (1.17-2.64) | .007 | |
| hENT1 expressions of IHC | | | | | |
| Weak or more/No | 1.01 (0.68-1.50) | .952 | | 1.24 (0.82-1.86) | .308 | |
| Moderate or strong (positive)/ No or weak (negative) | 0.78 (0.42-1.45) | .430 | | 1.01 (0.69-1.46) | .979 | |
| Strong/Moderate or less | 1.51 (0.81-2.83) | .200 | | 0.89 (0.49-1.62) | .704 | |
| DPD expressions of IHC | | | | | |
| Weak or more/No | 1.13 (0.78-1.84) | .530 | | 1.27 (0.87-1.86) | .207 | |
| Moderate or strong (positive)/ No or weak (negative) | 0.85 (0.56-1.30) | .445 | | 0.94 (0.61-1.45) | .794 | |
| Strong/Moderate or less | 0.86 (0.32-2.33) | .765 | | 0.54 (0.17-1.71) | .296 | |

DPD, dihydropyrimidine dehydrogenase; ECOG, Eastern Cooperative Oncology Group; GEM, gemcitabine; hENT1, human equilibrative nucleoside transporter-1; IHC, immunohistochemistry.
predicting the therapeutic effect of S-1 using a large-scale randomized clinical trial in the field of pancreatic cancer.

Elander et al revealed that high DPD expression was related to poor survival in a 5-FU/FA arm. They concluded that the high DPD expression in tumors was a negative prognostic biomarker and that hENT1 and DPD expressions might be useful to select either postoperative 5-FU/FA or GEM.26 These results were consistent with those of most published reports.16,25 In contrast, Sasako et al showed that the high DPD expression in tumors was associated with substantial benefit from adjuvant treatment in a large biomarker study in the field of gastric cancer.19 These inconsistent results between S-1 and 5-FU/FA may be caused by inhibition of DPD by gimeracil contained in S-1. In addition, thymidylate synthase (TS) is known as the primary target of fluoropyrimidines27 and has been shown to be a favorable biomarker related to prognosis in S-1-treated patients with pancreatic cancer.28 Further study is necessary to explore the role of TS expression in predicting the effects of adjuvant drugs with pancreatic cancer.

We used immunohistochemistry to evaluate the expression of hENT1 and DPD in the present study. Most studies using large-scale clinical test specimens evaluate gene expression using a tissue microarray (TMA).12-15,20 It remains possible that results were affected by the parts of the tumors that we evaluated tumor because pancreatic cancer tumors are very heterogeneous. In this regard, the use of immunohistochemistry in the present study allowed us to evaluate a broader range of tumors than TMA.

The present study has several limitations. We retrospectively designed this biomarker study after the completion of the final analysis of the JASPAC 01 study. Thus, not all patients permitted the examination of tumor samples; however, the collection rate was very high (86.5%) compared to similar studies using large-scale clinical test specimens. Moreover, the subset data were equivalent to the overall study population data of the JASPAC 01 study. All patients who enrolled in the JASPAC 01 study were East Asian. The pharmacokinetics and pharmacodynamics of anti–cancer drugs in European and North American patients and East Asian patients might differ due to genetic differences. A further prospective study is therefore needed to objectively validate the results of the present study because the hENT1 expression is not used in actual clinical practice, even following the publication of several studies that have shown that hENT1 is a useful biomarker in patients treated with GEM.12-18

In conclusion, the present study did not show the utility of the hENT1 and DPD expression levels as biomarkers for GEM and S-1, respectively, contrary to the hypothesis, which was based on the previous studies. In contrast, the median overall survival in the S-1-treated patients with low hENT1 expression was significantly better than that with high hENT1 expression and the low expression of hENT1 (weak or negative IHC staining) was a significant predictor for overall survival in the S-1 arm.

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CONFLICT OF INTEREST
KU received honoraria from Taiho. HN received research funding from Chugai Pharmaceutical. NB received honoraria from Taiho and Eli Lilly. AF received honoraria from Taiho, Eli Lilly Japan, Yakult Honsha and Daiichi Sankyo, and received rewards for an advisory role from Yakult Honsha. SN received research funding from Eisai. SH received a scholarship donation from Taiho. All remaining authors have declared no conflict of interest.

ORCID
Yukiyasu Okamura https://orcid.org/0000-0003-3384-2709
Hiroto Narimatsu https://orcid.org/0000-0002-0383-4911

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