Pancreatic ductal adenocarcinoma (PDAC) has been suggested to be a systemic disease at the time of diagnosis because of the exceedingly high rates of distant metastatic recurrence even after successful surgical resection of early-stage tumors. Therefore, a multimodal treatment of PDAC is required because surgical treatment alone does not greatly improve survival.

Chemoradiotherapy (CRT) before surgery for PDAC may provide for the early treatment of a micrometastatic disease, allowing for the identification of patients with metastatic disease at the time of reassessment, and increase the R0 resection rate, resulting in a reduced risk for local tumor recurrence.

Nevertheless, surgical resection is the only potentially curative technique for managing PDAC. R0 resection is a strong prognostic indicator for long-term patient survival.

With respect to the recommended treatment of the 3 groups, in group R, initial surgical resection is defined as the standard therapeutic strategy, but some reports have suggested that neoadjuvant CRT (NCRT) for R tumors is feasible because PDAC is a systemic disease. Even if R0 resection is achieved for an R tumor, 10% to 15% of them will exhibit an early recurrence;
thus, NCRT could help select patients who might not benefit from surgical resection. In the BR group, although there is no high-level evidence supporting treatment with NCRT, some institutions prefer an initial approach involving neoadjuvant therapy. In the UR group, NCRT is not usually used but it has been reported that UR tumors may be downstaged by CRT to allow for surgical resection. Consequently, there has been no consensus or clear evidence concerning the indication of NCRT for R, BR, and UR tumors.

Our institution has introduced gemcitabine-based CRT followed by surgery (gem-CRTS) for the treatment of PDAC since February 2005. Gemcitabine (20,20-difluoro-2-2-deoxycytidine analog) inhibits DNA replication and repair. Recently, intratumoral human equilibrative nucleoside transporter 1 (hENT1), which is the major transporter responsible for gemcitabine uptake into cells, has been reported as an important predictive marker of chemosensitivity for gemcitabine-based adjuvant chemotherapy (AC) for PDAC. We previously reported that hENT1 expression was an independent predictor of overall survival after gem-CRTS in patients with Union Internationale Contre le Cancer (UICC) T3 to T4 PDAC. However, there have been no previous reports describing the significance of gem-CRTS for PDAC according to resectability based on the NCCN guidelines, especially focusing on hENT1 expression.

The aim of the present study was to evaluate the efficacy of gem-CRTS for the treatment of PDAC regarding the 3 resectability groups (R, BR, and UR) defined by the NCCN pancreatic cancer guidelines (2010), with special attention to serum CA19-9 alternation and hENT1 expression.

MATERIALS AND METHODS

Between February 2005 and October 2010, 100 patients with PDAC who were diagnosed as having UICC-T3 and UICC-T4 tumors using multidetector computed tomography (MDCT) were enrolled for our gem-CRTS protocol. All patients were warned of the risks of treatment, especially concerning the possibility of developing distant metastases after gem-CRT treatment. They all gave their written informed consent for inclusion in the study. The diagnosis of pancreatic cancer was confirmed by means of cytological or histological analysis of biopsy specimens obtained using endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA). Patients were excluded when the tumor extension determined by MDCT was categorized as UICC-T1 or UICC-T2 and/or when they showed evident distant metastatic lesions. The study protocol was approved by the medical ethics committee of Mie University, and the study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

All patients underwent pretreatment examination using a 64-slice MDCT. Computed tomography (CT) was performed according to a defined pancreas protocol as 4-phasic contrast-enhanced MDCT with thin slices at intervals of 1 mm. In the present study, all of these 100 patients were reclassified into the 3 groups (R, BR, and UR) according to NCCN guidelines (2010) based on MDCT findings at the initial visit to our hospital. The CT criteria of the NCCN guidelines are as follows: R criteria, (1) no evidence of SMV and PV abutment, distortion, tumor thrombus, or venous encasement and (2) clear fat planes around the CA, hepatic artery, and SMA; BR criteria, (1) venous involvement of the PV/SMV demonstrating tumor abutment without impingement and narrowing of the lumen, encasement of the PV/SMV without encasement of the nearby arteries, or short-segment venous occlusion resulting from either tumor thrombus or encasement but with suitable vessel proximal and distal to the area of vessel involvement, allowing for safe resection and reconstruction, (2) gastroduodenal artery encasement up to the hepatic artery with either short-segment encasement or direct abutment of the hepatic artery without extension to the CA, and (3) tumor abutment of the SMA not exceeding greater than 180° of the circumference of the vessel wall; and UR criteria, (1) greater than 180° of SMA encasement, celiac involvement (any abutment of the head with a greater than 180° encasement of the body or tail), (2) unreconstructive PV/SMV occlusion, and (3) aortic invasion. On the basis of the objective CT criteria, the patients enrolled in our study were classified as follows: 14 patients with R, 44 with BR, and 42 with UR tumors.

Treatment Plan and Assessment of Gem-CRTS

Our treatment protocol for gem-CRTS has been reported previously. All patients were treated with 3-dimensional conformal radiotherapy using the 4-field box technique from directions that avoided exposure of the kidney, which was an organ at risk. Based on the CT images, the gross tumor volume, which included the main tumor and lymph nodes of more than 1 cm in diameter, was defined. The clinical target volume was defined as the gross tumor volume plus a 5-mm margin in all directions. The planning target volume was basically defined as the clinical target volume plus a 5-mm margin, and an additional 10-mm margin was added in the cranial-caudal direction. The total radiation dose delivered was 45 Gy in 25 fractions (5 fractions/wk).

Radioactive responses were determined by means of a comparison of pretreatment CT and post-CRT scans. Response was judged according to the Response Evaluation Criteria in Solid Tumors. We used serum CA19-9 levels as an index of response to gem-CRT. Serum CA19-9 levels were measured just before the initiation of gem-CRT (pre-CA19-9) and every 4 weeks thereafter. In the patients with obstructive jaundice, drainage was achieved using endoscopic retrograde biliary drainage, endoscopic nasobiliary drainage, or percutaneous transhepatic cholangio-drainage before gem-CRT. We compared the level of pre-CA19-9 and CA19-9 levels at that measured at the time of reassessment (post-CA19-9).

Indication of Resection, Surgical Procedure, and Postoperative Complications

At the time of reassessment, especially in the case of UR patients, we determined that curative-intent resection was possible when the following findings on MDCT were observed: no stenosis or change of shape in the celiac trunk and SMA as well as the absence of metastatic lesions in other distant organs. Intraoperatively, curative-intent resection was avoided when
distant metastatic disease was detected on histological examination of frozen sections of suspicious lesions and of distant lymph nodes, including paraaortal lymph nodes. Curative-intent resection was also avoided when the tumor was found to be appreciably locally advanced, showing unreconstructable PV/SMV occlusion even if an external iliac vein graft had been used and/or a severe tumor invasion around the SMA (which was impossible to dissect without a remnant tumor) was evident.

Pancreatecdoenectomy (PD) or distal pancreatectomy (DP) was performed as previously described.15 Resection and reconstruction of the PV/SMV were performed when the surgeon could not separate the pancreatic head or the uncinate process from these vessels without leaving gross tumor on the vessel. When limited involvement of the common hepatic artery was identified, a segmental resection of this vessel was performed with primary anastomosis. The patients who had an unresectable disease at surgery, which was usually due to the presence of distant metastasis, underwent surgical bypass as clinically indicated.

Postoperative complications including morbidity and mortality were graded according to the Clavien-Dindo classification.20 Postoperative pancreatic fistula, which is a complication specific to pancreatectomy, was graded according to the International Study Group on Pancreatic Fistula classification.21 Postoperative mortality was defined as all causes of death in the hospital.

**Results**

The types of pancreatectomy in the R, BR, and UR groups were PD in 4, 32, and 16 patients, respectively, as well as DP in 10 patients (R, 1; BR, 2; UR, 7) were found to have an unresectable disease owing to the presence of radiographically occult distant metastases (R, 1; BR, 5; UR, 13). The reasons that patients were inoperable in the R, BR, and UR groups were distant metastases in 3 (3/11, 27%), 5 (5/43, 12%), and 7 (7/40, 18%), respectively, and local tumor factors in 0, 0, and 6 (6/40, 15%), respectively. Among the 73 patients who presented for surgery, 10 patients (R, 1; BR, 2; UR, 7) were found to have an unresectable disease owing to the presence of radiographically occult distant metastases (R, 1; BR, 4; UR, 5) or local tumor factors (BR, 1; UR, 3), and thus, the curative-intent resection rate was 87.5% (7/8) in R patients, 94.7% (36/38) in BR patients, and 74.0% (20/27) in UR patients.

The flow of all 100 patients through the treatment protocol is illustrated in Figure 1. The gem-CRT was completed in all 14 (100%) of the R patients, in all 44 (100%) of the BR patients, and in 40 (95.2%) of the 42 UR patients. Two (2.0%) of the 100 patients could not complete gem-CRT because of a decline in performance status related to disease progression. During gem-CRT, grade 3 gastrointestinal toxicities occurred in 1 of the R patients and in 3 of the UR patients. Thirty-eight patients (R, 6; BR, 12; UR, 20) had grade 3 hematological toxicities, and 5 patients (R, 1; BR, 3; UR, 1) had grade 4 hematological toxicities. Three R patients and 1 BR patient who completed gem-CRT did not return to the hospital for surgery. Finally, 94 patients (R, 11; BR, 43; UR, 40) could be reassessed; 73 were operable (R, 8; BR, 38; UR, 27), and 21 were inoperable (R, 3; BR, 5; UR, 13). The reasons that patients were inoperable in the R, BR, and UR groups were distant metastases in 3 (3/11, 27%), 5 (5/43, 12%), and 7 (7/40, 18%), respectively, and local tumor factors in 0, 0, and 6 (6/40, 15%), respectively. Among the 73 patients who presented for surgery, 10 patients (R, 1; BR, 2; UR, 7) were found to have an unresectable disease owing to the presence of radiographically occult distant metastases (R, 1; BR, 4; UR, 5) or local tumor factors (BR, 1; UR, 3), and thus, the curative-intent resection rate was 87.5% (7/8) in R patients, 94.7% (36/38) in BR patients, and 74.0% (20/27) in UR patients.

The types of pancreatectomy in the R, BR, and UR groups were PD in 4, 32, and 16 patients, respectively, as well as DP in 3, 4, and 4 patients, respectively. The combined resection rate of the R and IV was significantly higher in the BR (32/36, 88.9%) and UR (19/20, 95.0%) groups, as compared with that in the R group (2/7, 28.6%) (P < 0.001). In 5 patients (3 in the BR and 2 in the UR group), an external iliac vein graft was used as an interpositional venous graft to reconstruct the PV/SMV. A combined resection of the celiac trunk was performed in 3 UR patients, and resection of the hepatic artery, in 2 BR and 2 UR patients. The R0 resection rate was significantly higher in R (7/7, 100%) and BR patients (32/36, 77.8%), as compared with that in UR patients (8/20, 40.0%) (P = 0.0023).

**Statistical Analyses**

In all patients who came for reassessment, the date of the initial treatment was chosen as the starting point for the measurement of survival time. Disease-free survival time was calculated in the patients that underwent resection, and defined as the time from the date of initial treatment to the date of first relapse or death. Survival was calculated using the Kaplan-Meier method and was compared between the groups using the log-rank test. The day of final follow-up was July 31, 2012, and there was no loss of follow-up. All variables were dichotomized for analyses. A multivariate analysis was performed using Cox proportional hazard model. Variables with a significance of P < 0.1 in the univariate analysis were entered into the multivariate analysis. Comparisons were performed using the χ² test with Yates correction in the univariate analysis. All statistical analyses were performed using the SPSS version 18 (SPSS Inc, Chicago, Ill) software. A P value less than 0.05 was considered statistically significant.
Patient characteristics and the outcomes of gem-CRT treatment are summarized in Table 1. There were no statistically significant differences in age and size of tumor before gem-CRT therapy among the 3 groups. According to the evaluation using MDCT before gem-CRT, all of the BR and UR patients had cancer involvement in large vessels but no R patient did. The response status after gem-CRT did not differ among the 3 groups. Although CA19-9 levels (median) decreased after gem-CRT in all 3 groups, the pre- and post-CRT CA19-9 levels did not differ among the 3 groups. The incidence of patients with a greater than 50% reduction in CA19-9 level was 27.3% (3/11) in the R, 53.5% (23/43) in the BR, and 35.0% (14/40) in the UR groups (these values did not differ significantly).

The cumulative survival curves for 94 patients in the 3 groups who were reassessed are shown in Figure 2A. The 3-year survival rates of R, BR, and UR patients were 60.6%, 27.4%, and 4.6%, respectively (R vs UR, \( P = 0.0115 \)).

The univariable and multivariable analyses of the effect of preoperative factors on survival time are summarized in Table 2. The statistically significant variables in the univariable analyses were the CA19-9 reduction rate (\( P = 0.0003 \)) and cancer involvement of a major artery (\( P = 0.0421 \)) in the BR group as well as sex (\( P = 0.0306 \)) in the UR group. The multivariable analysis indicated the CA19-9 reduction rate in the BR group as the single significant independent factor.

Postoperative complications (Clavien grades IIIa-V) occurred in 1 (14.3%) of 7 R patients, in 6 (16.7%) of 36 BR patients, and in 3 (15.0%) of 20 UR patients (Table 3). There were no significant differences in postoperative complications (Clavien grades IIIa–V) between the 3 groups. Postoperative 30-day mortality occurred in 2 patients owing to pneumonia (a BR patient who underwent PD) and sepsis (a UR patient who underwent DP) caused by grade C pancreatic fistula according to the International Study Group on Pancreatic Fistula criteria.

When we compared pathological factors according to resectability groups (Table 4), a high response was observed in 4 R patients (50%), 9 BR patients (25%), and 3 UR patients (15%). The rate of positive hENT1 expression was almost the same in each of the 3 groups, as follows: 67% in the R, 72% in the BR, and 60% in the UR group. There were no significant differences in the degree of lymphatic and venous invasion, whereas for nerve invasion, the incidence of ne1-3 was significantly higher in the UR relative with those in the R and BR groups (\( P = 0.022 \)).

The cumulative survival curves for 63 patients in the 3 groups who completed the gem-CRTS treatment are shown in Figure 3A. The 3-year survival rates of the R, BR, and UR patients were 83.3%, 33.0%, and 7.8%, respectively (R vs BR, \( P = 0.0208 \); R vs UR, \( P = 0.0022 \)). The disease-free survival curves for the 3 groups after initial treatment are presented in Figure 3B. The 3-year disease-free survival rates for R, BR, and UR patients were 83.3%, 31.8%, and 7.8%, respectively (without any statistical difference).

Table 5 shows the univariable and multivariable analyses of preoperative and postoperative factors regarding survival time
TABLE 1. Characteristics of Patients Enrolled for the Gem-CRT Protocol and the Outcome of Gem-CRT

| Variable | R (n = 14) | BR (n = 44) | UR (n = 42) | P      |
|----------|-----------|------------|------------|--------|
| **Age, mean (SD), y** | 66.4 (9.9) | 68.8 (9.1) | 66.1 (8.8) | 0.8142 |
| **Size of tumor before gem-CRT, mean (SD), cm** | 3.1 (1.2) | 3.0 (0.9) | 3.6 (1.1) | 0.1562 |
| **Cancer involvement of large vessels, n (%)** | 0 (0) | 44 (100) | 42 (100) | 0.8142 |
| PV/SMV, n | 0 | 37 | 32 | 0.1562 |
| SMA, n | 0 | 8 | 27 | 0.1562 |
| Ceriac artery, n | 0 | 2 | 29 | 0.1562 |
| Hepatic artery, n | 0 | 11 | 20 | 0.1562 |
| IVC, aorta, n | 0 | 0 | 2 | 0.1562 |
| **Gem-CRT completion rate, n (%)** | 14/14 (100) | 44/44 (100) | 40/42 (95) | 0.3064 |
| Did not return to hospital, n | 3 | 1 | 0 | 0.3064 |
| Reassessed cases, n (%) | 11 (78.6) | 43 (97.7) | 40 (95.2) | 0.3064 |
| **Response of gem-CRT** | | | | 0.5558 |
| CR | 0 | 0 | 0 | 0.5558 |
| PR | 1 (1) | 5 (5) | 2 (2) | 0.5558 |
| SD | 7 (6) | 31 (29) | 30 (18) | 0.5558 |
| PD | 3 (0) | 7 (2) | 8 (0) | 0.5558 |
| **Distant metastasis after gem-CRT, n (%)** | 3/11 (27) | 5/43 (12) | 7/40 (18) | 0.4167 |
| CA19-9 levels, median, U/mL | | | | 0.2467 |
| Pre-CAN-9 | 95.1 | 275.2 | 160.6 | 0.2467 |
| Post-CAN-9 | 32.0 | 77.7 | 129.2 | 0.2467 |
| **Degree of reduction rate in CA19-9 level** | | | | 0.1295 |
| ≥50% | 3 (2) | 23 (23) | 14 (9) | 0.1295 |
| <50% | 8 (5) | 20 (13) | 26 (11) | 0.1295 |

*The numbers of resected cases are shown in parentheses.

CR indicates complete response; IVC, inferior vena cava; PR, partial response; SD, stable disease; PD, progressive disease.

FIGURE 2. Survival curves after initial treatment in reassessed patients. A, Cumulative survival curves according to the 3 groups in 94 patients who were reassessed. B, Cumulative survival curves according to the CA19-9 reduction rate in 43 BR patients who were reassessed.
after gem-CRTS (excluding R patients because 6 of the 7 patients remain alive). The statistically significant factors in the univariable analyses in BR patients were the CA19-9 reduction rate, status of the surgical margin, histological effect of gem-CRT, and nerve invasion; those in UR patients were the CA19-9 reduction rate and hENT1 expression. The multivariable analyses indicated the status of the surgical margin in the BR group and positive hENT1 expression in the UR group as the single independent significant factors.

We compared survival curves according to hENT1 expression in BR (Fig. 3C) and UR patients (Fig. 3D). The 3-year survival rate was not significantly different between positive and negative hENT1 expressions (37.2% vs 22.2%) in the BR group (median survival time [MST], 24.2 months vs 12.9 months; hENT1 expression in the UR group as the single independent significant factors.

### TABLE 2. Univariable and Multivariable Analyses of the Effect of Preoperative Factors on Survival Time in Reassessed Cases

| Variable                                      | R (n = 11)* | BR (n = 43) | UR (n = 40)* |
|-----------------------------------------------|------------|-------------|--------------|
|                                               | Univariable, P | Univariable, P | Multivariable, HR (95% CI) |
| Sex                                           | 0.3278     | 0.4760      | 0.0306       |
| Age                                           | 0.9919     | 0.2778      | 0.9636       |
| Tumor location                                | 0.2853     | 0.1628      | 0.4820       |
| Tumor size                                    | 0.5181     | 0.8812      | 0.8062       |
| Cancer involvement of PV/SMV                  |             |             |              |
| Positive vs negative                          | 0.0421     | 1.430 (0.599–3.415) | 0.1668       |
| Cancer involvement of major artery            |             |             |              |
| Positive vs negative                          |             |             |              |
| RECIST                                        | 0.1241     | 0.6141      |              |
| Reduction rate in serum CA19-9 level          | 0.8658     | 0.0003      | 3.445 (1.559–7.613) | 0.4003 |

Major artery includes the SMA and/or CA.

*Multivariable analyses were not assessed.

CI indicates confidence interval; HR, hazard ratio; RECIST, Response Evaluation Criteria in Solid Tumors.

### TABLE 3. Postoperative Mortality and Morbidity

|                  | R (n = 7) | BR (n = 36) | UR (n = 20) |
|------------------|----------|------------|------------|
|                  | PD (n = 4) | DP (n = 3) | PD (n = 32) | DP (n = 4) | PD (n = 16) | DP (n = 4) |
| Clavien-Dindo classification, n |          |            |            |            |            |
| Grade IIa        | 1        | 4          | 1          |            |            |
| Grade IIb        | 1        | 1          | 1          |            |            |
| Grade V          | 1        | 1          | 1          |            |            |
| ISGPF classification, n |        |            |            |            |            |
| Grade A          |          |            | 1          |            |            |
| Grade B          |          |            |            | 1          |            |
| Grade C          | 1        | 1          | 1          |            |            |
| Complications, n |          |            |            |            |            |
| Abdominal abscess | 1        |            |            |            |            |
| Intractable ascites | 3        |            |            |            |            |
| Pneumonia        | 2        |            |            |            |            |
| Gastric hemorrhagic ulcer | 1 |            |            |            |            |
| Anastomotic leakage | 2        |            |            |            |            |
| Thrombosis in the IVC |      |            |            |            |            |
| Sepsis           | 1        |            |            |            |            |

ISGPF indicates International Study Group on Pancreatic Fistula; IVC, inferior vena cava.
TABLE 4. Pathological Findings Regarding the Resectability Groups

| Variable                        | R (n = 7) | BR (n = 36) | UR (n = 20) | P      |
|---------------------------------|-----------|-------------|-------------|--------|
| Histological effect of CRT, n   |           |             |             |        |
| Grade I                         | 0         | 15          | 4           | 0.0877 |
| Grade IIa                       | 3         | 12          | 13          |        |
| Grade IIb                       | 2         | 8           | 3           |        |
| Grades III and IV               | 2         | 1           | 0           |        |
| hENT1 expression                |           |             |             |        |
| Positive, n (%)                 | 4 (66.7)  | 26 (72.2)   | 12 (60.0)   | 0.6065 |
| Negative, n                     | 2         | 10          | 8           |        |
| Ly                              |           |             |             |        |
| Ly0, n                          | 2         | 5           | 2           | 0.479  |
| Ly1–3, n (%)                    | 5 (71.4)  | 31 (86.1)   | 18 (90.0)   |        |
| V                               |           |             |             |        |
| V0, n                           | 5         | 15          | 9           | 0.350  |
| V1–3, n (%)                     | 2 (28.6)  | 21 (58.3)   | 11 (55.0)   |        |
| Nerve invasion                  |           |             |             |        |
| Ne0, n                          | 2         | 11          | 0           | 0.022  |
| Ne1–3, n (%)                    | 5 (71.4)  | 25 (69.4)   | 20 (100)    |        |

Ly indicates degree of lymphatic invasion; V, degree of venous invasion; Ne, degree of intrapancreatic nerve invasion.

FIGURE 3. Survival curves after initial treatment in patients who completed gem-CRTS. A, Cumulative survival curves for the R, BR, and locally UR groups (63 patients in total) who completed gem-CRTS (resected cases). B, Disease-free survival curves for the 3 groups involving the 63 patients who completed gem-CRTS (resected cases). C, Cumulative survival curves according to hENT1 expression in the 36 BR patients who completed gem-CRTS. D, Cumulative survival curves according to hENT1 expression in the 20 UR patients who completed gem-CRTS.
whereas in the UR group, there was a significant difference (11.9% vs 0%; MST, 22.8 months vs 10.6 months; \(P = 0.003\)).

Table 6 shows the CA19-9 reduction rate, histological response, status of AC, and sites of tumor recurrence according to hENT1 expression among the 3 groups. In the R group, we excluded 1 patient from the examination of hENT1 expression because the patient had no residual tumor in the resected specimen after gem-CRTs. In the BR patients, the CA19-9 reduction rate and histological response, which were considered as indicators of the gem-CRT effect, showed significantly higher incidences of CA19-9 reduction greater than 50%. In addition, there was a

TABLE 5. Univariable and Multivariable Analyses of Preoperative and Postoperative Factors on Survival Time After Gem-CRTs

| Variable | BR (n = 36) | Multivariable, HR (95% CI) | UR (n = 20) | Multivariable, HR (95% CI) |
|----------|------------|----------------------------|------------|----------------------------|
| Sex      | Male vs female patients | Univariable, \(P\) | Multivariable, \(P\) | Univariable, \(P\) |
| Age      | <65 vs ≥65 y | 0.6543 | 0.4069 |
| Tumor location | Head vs body/tail | 0.3544 | 0.5325 |
| Cancer involvement of PV/SMV | Positive vs negative | 0.3945 | 0.3893 |
| Cancer involvement of major artery | Positive vs negative | 0.3436 | 0.3045 |
| Reduction rate in serum CA19-9 level ≥50% vs <50% | | 0.0145 | 1.360 (0.534–3.464) | 0.0310 | 3.778 (0.640–22.285) |
| Status of surgical margin | R0 vs R1 and R2 | <0.001 | 5.204 (1.547–17.506) | 0.3448 |
| Histological effect of gem-CRT | Grades I and IIa vs Grades IIb and III | 0.0393 | 1.965 (0.620–6.230) | 0.6530 |
| hENT1 expression | Positive vs negative | 0.0688 | 2.585 (0.743–8.989) | <0.001 | 18.515 (2.148–159.595) |
| Ne | Ne0 vs ne1–3 | 0.0378 | 2.847 (0.745–10.878) |

\(Ne\) indicates grade of intrapancreatic nerve invasion.

\(P = 0.0688\), whereas in the UR group, there was a significant difference (11.9% vs 0%; MST, 22.8 months vs 10.6 months; \(P = 0.003\)).

Table 6 shows the CA19-9 reduction rate, histological response, status of AC, and sites of tumor recurrence according to hENT1 expression among the 3 groups. In the R group, we excluded 1 patient from the examination of hENT1 expression because the patient had no residual tumor in the resected specimen after gem-CRTs. In the BR patients, the CA19-9 reduction rate and histological response, which were considered as indicators of the gem-CRT effect, showed significantly higher incidences of CA19-9 reduction greater than 50%. In addition, there was a

TABLE 6. CA19-9 Reduction Rates, Histological Response, Status of Adjuvant Chemotherapy, and Sites of Tumor Recurrence According to hENT1 Expression Among the 3 Groups

| Variable | R (n = 6)* | BR (n = 36) | UR (n = 20) |
|----------|------------|------------|------------|
| CA19-9 ≥50%, n (%)† | hENT1+ (n = 4) | hENT1− (n = 2) | P | hENT1+ (n = 26) | hENT1− (n = 10) | P | hENT1+ (n = 12) | hENT1− (n = 8) | P |
| 1 (25.0) | 1 (50.0) | 0.541 | 20 (77.0) | 3 (30.0) | 0.025 | 6 (50.0) | 3 (37.5) | 0.582 |
| High responder, n (%) | 2 (50.0) | 1 (50.0) | 0.541 | 9 (34.6) | 0 (0) | 0.011 | 2 (16.6) | 1 (12.5) | 0.812 |
| AC commenced, n (%) | 2 (50.0) | 1 (50.0) | 1 | 19 (73.1) | 7 (70.0) | 0.820 | 11 (91.7) | 4 (50.0) | 0.110 |
| AC completed, n (%) | 2 (50.0) | 1 (50.0) | 1 | 13 (50.0) | 2 (20.0) | 0.210 | 8 (66.7) | 0 (0) | 0.005 |
| Recurrence, n (%) | 2 (50.0) | 0 (0) | 0.76 | 16 (61.5) | 7 (70.0) | 0.93 | 9 (75.0) | 6 (75.0) | 0.91 |
| Local, n | 1 | 0 | 4 | 0 | 2 | 1 |
| Metastasis, n | 1 | 0 | 14 | 7 | 7 | 5 |
| Lung | 1 | 0 | 6 | 2 | 1 | 2 |
| Liver | 0 | 0 | 10 | 2 | 4 | 3 |
| Peritoneal | 0 | 0 | 3 | 2 | 5 | 0 |
| Other | 0 | 0 | 2 | 1 | 0 | 0 |

*One patient was excluded because the patient had no residual tumor in the resected specimen after gem-CRTs.
†Reduction rate in serum CA19-9 level ≥50% after gem-CRT; AC, gemcitabine-based AC.
‡Including 3 cases with remnant pancreas.
higher rate of positive hENT1 expression than negative hENT1 expression (77% vs 30%, respectively \( P = 0.025 \), and 35% vs 0%, respectively \( P = 0.011 \)). In R and UR patients, however, there was no significant difference between positive and negative hENT1 expressions. It was possible to commence the planned gem-AC in 44 patients, but the remaining 18 patients could not be treated owing to a prolonged recovery time. Although the hENT1 expression did not influence the commencement of gem-AC among the 3 groups because there was no significant difference in the incidence of patients who commenced gem-AC, it significantly influenced the gem-AC completion rate in UR patients (66.7% with positive expression vs 0% with negative expression, \( P = 0.005 \)). The incidences of local recurrence and distant metastasis were not significantly different between the patients with positive and negative hENT1 expression in each group. When we compared the incidences of the CA19-9 reduction rate of greater than 50.0% in all 62 patients and in patients selected and treated with gem-AC according to hENT1 expression, there was a significantly higher positive expression than negative expression in the former group (64.3% [27/42] vs 35.0% [7/20] \( P = 0.030 \) and 54.8% [23/42] vs 15.0% [3/20] \( P = 0.003 \)). Furthermore, the 15 BR patients who completed gem-AC survived longer than the 21 who did not complete gem-AC; the MSTS were 24.4 and 18.8 months, respectively (\( P = 0.014 \)). Eight UR patients who completed gem-AC survived longer than the 12 who did not complete gem-AC; the MSTS were 26.8 and 10.8 months, respectively (\( P = 0.0002 \)).

**DISCUSSION**

Unexpectedly, at the time of reassessment in our study, distant metastases had occurred at a similar frequency among the 3 groups, as follows: 27% in the R group, 12% in the BR group, and 18% in UR group. To our knowledge, there have been no reports regarding the frequency of occurrence of distant metastasis after completion of gem-CRT according to resectability based on the NCCN guidelines. Previous studies have reported that the occurrence rate of distant metastasis at the time of reassessment after NCRT was 12.8% in BR patients and 12.5% in potentially resectable tumors (including R and BR tumors). However, there have been no reports concerning UR. Our results suggest that PDAC is a systemic disease regardless of resectability status. A subgroup analysis carried out in the CONKO-001 randomized controlled trial regarding AC after curative-intent resection1 also suggested that PDAC is a systemic disease even in early-stage tumors at the time of diagnosis. This was because the median disease-free survival time among patients with T1 to T2 tumors in the gemcitabine group (12.9 months), was almost the same as that among patients with T3 to T4 tumors in the gemcitabine group (12.9 months), was substantially shorter than that in the gemcitabine group (10.0 vs 48.2 months). One of the advantages of NCRT is the identification of a subset of patients for whom resection will not offer a survival benefit. In fact, all of the R patients that underwent resection in our study did not experience recurrence within 2 years after gem-CRTS treatment. Although NCRT is recommended for the treatment of BR tumors as an option,3 it may also be recommended for R tumors.

Although CA19-9 has been accepted as a measure of pancreatic cancer burden, the role of CA19-9 in the evaluation of patients with NCRT before planned surgical resection has not been well evaluated. Recently, there have been 2 studies that have underscored CA19-9 as a marker of resectability and survival in patients with potentially R PDAC treated with NCRT. One of these studies26 indicated that a pre–CA19-9 level of less than 37 U/mL had a positive predictive value of 86% for completing PD but a negative predictive value of 33%; in addition, a post-CA19-9 level of less than 61 U/mL had a positive predictive value of 93% and a negative predictive value of 28%. The other study25 used more complicated criteria by dividing the patients into 3 categories (I, increased; MD, modestly decreased; SD, substantially decreased) and using pre–CA19-9 level and post–CA19-9 reduction rate as end points; the authors suggested that alteration in CA19-9 status was a single independent factor associated with prognosis. In the present study, the multivariable analysis of the effect of preoperative factors on survival time in reassessed cases indicated that a CA19-9 reduction rate of more than 50% was the single significant prognostic factor in BR patients but not in R and UR patients. In our study, all of the 15 patients (3 in the R group, 5 in the BR group, and 7 in the UR group) who developed distant metastases at reassessment did not show a CA19-9 reduction rate of greater than 50%, suggesting that CA19-9 reduction rate is associated with the systematic progression of PDAC.

Several previous studies have indicated that margin resection status is a very important prognostic factor and that the survival benefits of R1 resection may be comparable to palliative CRT without surgery.27 There have been few studies that have evaluated the R0 resection rates in BR according to the NCCN guidelines, and we could find only 2 studies that had evaluated the R0 resection rates in BR patients. One of these studies28 emphasized the effect of NCRT on the R0 resection rate by retrospectively comparing patients that had and had not undergone NCRT for BR tumors; the R0 resection rate was significantly higher in patients who had received NCRT than in those who had not (59% vs 11%). The other study, which was Japanese,29 compared the R0 resection rate between R (n = 109) and BR (n = 24) patients who were retrospectively classified according to NCCN guidelines and reported rates of 81% and 71%, respectively. In our study, the R0 resection rate was 100% in R, 78% in BR, and 40% in UR patients. Our results suggested that gem-CRT increased the R0 resection rate in R and BR patients and moreover, that the R0 resection rate was an independent prognostic risk factor in BR patients. As for the R0 resection rate in UR patients who underwent curative-intent resection after CRT, there have been no reports because UR patients are usually not candidates for resection. Only a few studies12–14 have reported that 8.6% to 32% of locally advanced patients (including BR and UR patients) who received CRT had undergone resection. To the best of our knowledge, the present study is the first to have evaluated the R0 resection rate by performing curative-intent resection after gem-CRT in UR patients. Among 42 UR patients enrolled for gem-CRTS, curative-intent resection could be performed in 20 (48%), of whom only 8 (40%) had undergone an R0 resection.

The rate of high responders to gem-CRT has been getting worse in line with resectability (57% in R, 25% in BR, and 15% in UR patients). Although the mechanism for resistance to CRT has not been fully explored, it has been reported that pancreatic cancer stem cells are a fundamental reason for this resistance.30,31 A recent study32 has revealed that chemoradioresistant pancreatic cancer cells are rich in stem-cell–like tumor cells and undergo epithelial-mesenchymal transition (EMT). It is known that N-cadherin is associated with a high invasiveness potential in cancer. A previous study33 has also demonstrated that overexpression of N-cadherin in PDAC, which was significantly correlated with the degree of nerve invasion, was involved in EMT. In the present study, the degree of nerve invasion was significantly higher in UR than in R and BR patients. Based on these findings, we hypothesize that pancreatic cancer cells in UR patients had become chemoradioresistant by undergoing EMT.
Several previous reports have demonstrated that hENT1 expression in the resected specimen is a significant predictive marker of chemosensitivity for gemcitabine-based AC in PDAC.\textsuperscript{15,13,36} As for neoadjuvant therapy, we could find only 2 previous reports, 1 of which was ours, that have described the relationship between hENT1 expression and prognosis for PDAC. Our previous study\textsuperscript{16} demonstrated that hENT1 expression was the independent predictor of overall survival after gem-CRTS in 55 patients with UICC-T3 to UICC-T4 PDAC. On the contrary, the other study\textsuperscript{37} found that hENT1 expression was not associated with prognosis in 63 patients who underwent gem-CRTS. The significant difference between the 2 studies involving gem-CRTS was the type of AC, gem-AC in the former and postoperative liver perfusion chemotheraphy using continuous infusion of 5-fluorouracil in the latter. In our previous study,\textsuperscript{16} the percentage of patients who completed gem-AC had significantly lower negative hENT1 expression than positive expression, and in addition, the patients who completed gem-AC had a longer MST than those who did not complete gem-AC (24.9 vs 12.8 months). In the present study, the survival rate of BR patients did not differ significantly between those with positive and negative hENT1 expression, whereas UR patients with positive hENT1 expression had a significantly higher survival rate. Furthermore, the rate of completion of gem-AC treatment in UR patients was significantly higher in those with positive hENT1 expression than in those with negative expression (66.7% vs 0%), whereas in BR patients, there was no significant difference between the 2 groups. These results suggested that the status of hENT1 expression highly influenced the gem-AC completion rate, which in turn affected patient survival.

Pretreatment evaluation of hENT1 expression in pancreatic cancer tissue can be beneficial in predicting the efficacy of gemcitabine therapy before initial treatment. The EUS-FNA specimens that we used for cytological/histological diagnosis might be suitable for evaluating hENT1 expression; however, the analysis of hENT1 expression in the pancreatic tumor tissue taken by EUS-FNA has not been established. Recently, we evaluated the availability of EUS-FNA samples for hENT1 expression and compared the status of hENT1 expression in the resected specimen in the 55 patients with PDAC treated with gem-CRTS.\textsuperscript{38} Among the 55 patients, only 23 (41.8%) who were histologically diagnosed as having PDAC could be evaluated for hENT1 expression in the EUS-FNA samples, positive for hENT1 expression in 16 (69.6%) samples and negative for hENT1 expression in 7 (30.4%) samples. The expression of hENT1 in 87% of EUS-FNA samples was identical with that in resected specimens after gem-CRT. The 16 patients with positive hENT1 expression in the EUS-FNA samples had significantly longer overall and recurrence-free survival rates than the 7 with negative hENT1 expression (2-year survival and recurrence-free survival rates, 67.5% and 29.2%, respectively, vs 35.7% and 0%, respectively). Our data provide the evidence that intratumoral hENT1 expression in EUS-FNA samples can be used to predict the treatment outcome before gem-CRT. However, improvement in the rate of acquisition of specimens by EUS-FNB and further modification of the protocol for the assay of hENT1 are needed.

In conclusion, a CA19-9 reduction rate of more than 50% after gem-CRT and R0 status were the significant prognostic factors in BR PDAC. Positive expression of hENT1 in the resected specimen was the significant prognostic factor in UR PDAC. We consider that our gem-CRTS protocol, even for locally UR PDAC, allows for the identification of candidates for aggressive resection at the time of reassessment, thus facilitating an increase in the R0 resection rate and improving prognosis in patients with positive hENT1 expression.

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