Statistical Analysis

The Student t test, Mann-Whitney U test, and the χ^2 test were used to compare the baseline characteristics of the treatment group and to compare the results of endoscopic biopsy and clinicopathological factors of the 2 groups. Overall survival was calculated from the date of neoadjuvant or definitive CRT to the occurrence of the event or to the last known date of follow-up. Actual survival was calculated by the Kaplan-Meier method and evaluated statistically by the log-rank test. A P value less than 0.05 was considered to reflect statistical significance. These analyses were carried out using the StatView J5.0 software package (Abacus Concepts, Berkeley, CA).

RESULTS

Correlation Between Endoscopic Biopsy Findings After Neoadjuvant CRT and Pathological and Survival Outcomes

Table 2 lists the characteristics of patients who received neoadjuvant CRT. Of 123 patients, the biopsy obtained after neoadjuvant CRT was negative for malignancy in 61 (50.0%) and positive in 62 (50.0%). There was a significant association between the histopathological findings of endoscopic biopsy and pathological tumor depth and tumor regression. Positive biopsy correlated significantly with minor histopathological tumor regression (grade 1), whereas negative biopsy correlated significantly with major histopathological tumor regression (grade 2 or 3). The sensitivity and specificity of endoscopic biopsy after neoadjuvant CRT in predicting pCR were 58.9% (56 of 95) and 78.6% (22 of 28), respectively. The positive and negative predictive values were 90.3% (56 of 62) and 36.1% (22 of 61), respectively. The findings on endoscopic biopsy predicted the pathological nodal status. Lymph node involvement was more frequent in patients with positive biopsy than in those with negative biopsy (62.9% vs 37.7%, $P = 0.005$).

Patients who received neoadjuvant CRT and later had negative biopsy showed significantly better survival than their counterparts with positive biopsy (5-year survival rate: 48.3% vs 21.8%, $P = 0.006$, Fig. 1). Of the 123 patients who received neoadjuvant CRT, recurrence was observed in 69 (56.1%) patients during the study period. Local recurrence was observed in 18 (14.6%) patients, lymphatic recurrence in 32 (26.0%) patients, and distant metastasis in 27 (22.0%) patients.

Correlation Between Endoscopic Biopsy Findings During and After Definitive CRT and Survival Rate

Of 66 patients who received definitive CRT, 32 (48.5%) had negative biopsy for malignancy whereas 34 (51.5%) had positive biopsy for malignancy at the dose of irradiation used in neoadjuvant setting (40 Gy) (Table 3). All but 2 of the 32 patients with negative biopsy at 40 Gy showed negative biopsy at the time of completion of definitive CRT, and 25 patients achieved clinical CR. On the contrary, 14 of the 34 patients with positive biopsy at 40 Gy improved to negative biopsy at the time of completion of definitive CRT, and 12 of these patients achieved clinical CR. Thus, the incidence of clinical CR after definitive CRT was significantly higher in patients with negative biopsy (78.1%) than in their counterparts with positive biopsy (35.3%, $P = 0.002$). In addition, the recurrence rate was significantly lower in clinical CR patients with negative biopsy after CRT (24%, 6 of 25) than in their counterparts with positive biopsy (67%, 8 of 12, $P = 0.0002$). Of the 37 patients who achieved clinical CR after definitive CRT, local recurrence was observed in 10 (27.0%) patients, lymphatic recurrence in 3 (8.1%) patients, and distant metastasis in 5 (13.5%) patients.

In the definitive CRT group, the 3-year survival rate of patients with negative biopsy at 40 Gy (57.0%) was significantly better than that of the patients with positive biopsy at 40 Gy (22.5%, $P = 0.0008$, Fig. 2). We also analyzed the survival data according to the results of endoscopic biopsy at the time of 40 Gy CRT and completion of definitive CRT (Fig. 3). Both the results of endoscopic biopsy at the time of CRT and at completion of definitive CRT influenced the survival rate.

TABLE 2. Clinicopathological Characteristics of Patients Who Underwent Neoadjuvant Chemoradiotherapy Followed by Surgery

	Results of Biopsy Obtained After Neoadjuvant CRT (40 Gy)		
	Negative	Positive	P value
n	61	62	
Mean age (years)	61.5	60.4	0.475
Gender (male/female)	52/9	55/7	0.568
Pretherapy tumor depth			
cT2	9 (15)	10 (16)	0.402
cT3	17 (28)	22 (36)	
cT4	35 (57)	30 (48)	
Pretherapy nodal status			
cN0	17 (28)	19 (31)	0.735
cN1	44 (72)	43 (69)	
Mean radiation dose (Gy)	40.0	40.0	0.541
Pathological tumor depth			
pT0	22 (36)	6 (10)	<0.001
pT1	5 (8)	6 (10)	
pT2	15 (24)	12 (19)	
pT3	14 (24)	22 (35)	
pT4	5 (8)	16 (26)	
Lymph node involvement			
pN0	38 (62)	23 (37)	0.005
pN1	23 (38)	39 (63)	
Clinical response			
CR	12 (20)	0 (0)	<0.001
PR	42 (69)	44 (71)	
NC/PD	7 (11)	18 (29)	
Pathological response			
Grade 3 (pCR)	22 (36)	6 (10)	<0.001
Grade 2	27 (44)	24 (39)	
Grade 1	12 (20)	32 (51)	

CR indicates complete response; NC/PD, no change or progressive disease; PR, partial response.

DISCUSSION

Both neoadjuvant CRT followed by surgery and definitive CRT have been recognized as curative treatment options for locally advanced esophageal cancers. Although a significant survival advantage has not been established for each therapeutic option, patients who show good response to CRT are considered to show a favorable prognosis. In fact, previous studies showed that the extent of

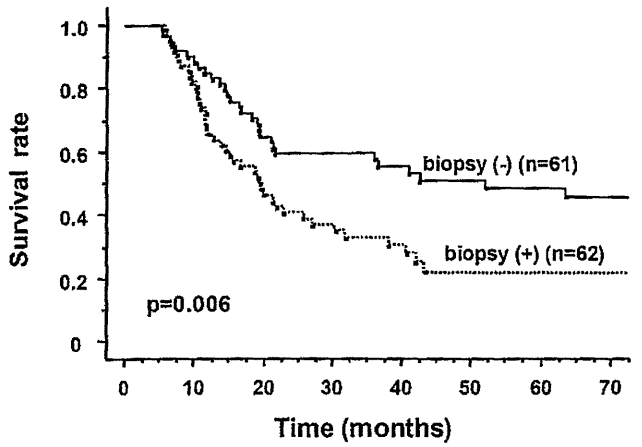

FIGURE 1. Overall survival rate in 123 patients with esophageal cancers who received neoadjuvant CRT followed by surgery, according to the results of endoscopic biopsy after induction CRT (40 Gy). The overall survival rate was significantly better in patients with negative biopsy than in those with positive biopsy.

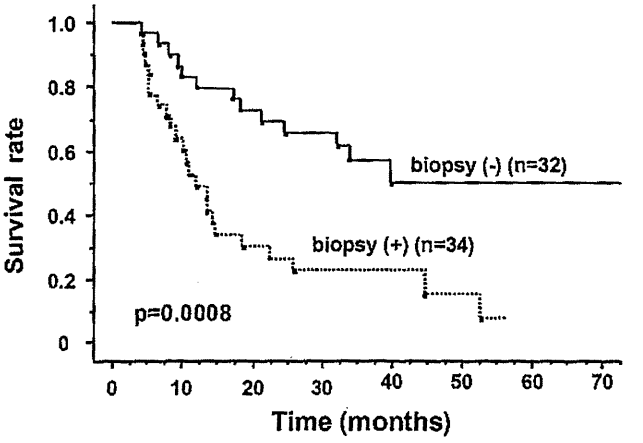

FIGURE 2. Overall survival rate in 66 patients with esophageal cancers who received definitive CRT, according to the result of endoscopic biopsy at the time of 40 Gy during the course of definitive CRT. The overall survival rate was significantly better in patients with negative biopsy than in those with positive biopsy.

TABLE 3. Characteristics of Patients Who Underwent Definitive Chemoradiotherapy

	Results of Biopsy Taken at Time of 40 Gy During Definitive CRT		
	Negative	Positive	*P* value
n	32	34	
Mean age (years)	65.2	66.8	0.483
Gender (male/female)	29/3	29/5	0.507
Pretherapy tumor depth			
cT1	13 (41)	7 (21)	0.088
cT2	3 (9)	5 (15)	
cT3	10 (31)	10 (29)	
cT4	6 (19)	12 (35)	
Pretherapy nodal status			
cN0	16 (50)	16 (47)	0.811
cN1	16 (50)	18 (53)	
Pretherapy stage			
I	12 (38)	7 (21)	0.575
II	3 (9)	7 (21)	
III	9 (28)	13 (37)	
IV	8 (25)	7 (21)	
Mean radiation dose (Gy)	60.6	62.6	0.091
Biopsy results after definitive CRT			
Negative	30 (94)	14 (41)	<0.001
Positive	2 (6)	20 (59)	
Clinical response after definitive CRT			
CR	25 (78)	12 (35)	0.002
PR	4 (13)	12 (35)	
NC/PD	3 (9)	10 (30)	

CR indicates complete response; NC/PD, no change or progressive disease; PR, partial response.

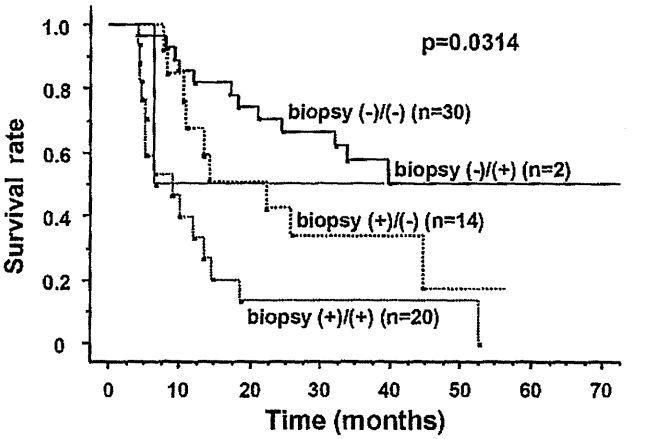

FIGURE 3. Overall survival rate in 66 patients with esophageal cancers who received definitive CRT, according to the results of endoscopic biopsy at the time of 40 Gy (represented by the first plus and minus sign after "biopsy") and at the time of completion of definitive CRT (represented by the second plus and minus sign after "biopsy").

histopathological tumor regression, especially pathological CR, correlated with improved prognosis.[17–19,39,40] Thus, the early detection of those patients who achieve good response to CRT is necessary so as to individualize therapy based on the response to induction therapy. In the present study, we investigated the prognostic significance of endoscopic biopsy findings at the dose of irradiation used in neoadjuvant setting (40 Gy), and we found that the findings of endoscopic biopsy at 40 Gy correlated with pathological tumor regression in neoadjuvant CRT and with clinical response in definitive CRT, and that such findings can predict the survival of patients who undergo CRT with and without surgery.

In neoadjuvant CRT, several studies investigated previously the use of endoscopic biopsy in predicting the pathological response to

neoadjuvant treatment.[27,32–35] However, these studies suggested that endoscopic biopsy findings were not useful in predicting pathological CR that would otherwise allow avoidance of surgical resection in patients with pathological CR. In the above studies, 70% to 80% of patients showed negative biopsy after neoadjuvant CRT, but pathological CR was observed in only 23% to 63% of patients with negative biopsy and the majority of those patients actually had residual cancer at resection. In our study, the endoscopic biopsy findings correlated significantly with histopathological tumor regression and survival after surgery. However, in agreement with previous studies, the biopsy findings were unreliable in predicting pathological CR, with pathological CR being observed in only 36% of patients with negative biopsy. The reason for this result is not clear at present but could be related to the fact that endoscopic biopsy can only detect cancer cells in the superficial mucosal layer. We reported previously that after CRT, few residual cancer cells were still present in deeper layers of the esophageal wall, such as the submucosal layer and muscularis propria.[41] These cancer cells can be missed even if endoscopic biopsy was taken accurately.

On the contrary, for definitive CRT, there is little or no information on the value of endoscopic biopsy during the course of radiotherapy. In the study of Kim et al,[35] which included examination of endoscopic biopsy samples from 94 patients with resectable esophageal cancer after neoadjuvant CRT (48 Gy), 27 of the 94 patients received a second course of CRT of up to 60 Gy as definitive CRT, but the value of endoscopic biopsy was not investigated in those patients who received definitive CRT. Our study demonstrated that the findings of endoscopic biopsy performed during the course of definitive CRT, at the time of 40 Gy irradiation, could predict the clinical response and survival after completion of definitive CRT. These results suggest that the findings of endoscopic biopsy conducted during the course of CRT are useful for selection of therapy after induction CRT, surgery, or completion of definitive CRT. In definitive CRT, the majority of patients whose biopsy results at the time of 40 Gy irradiation were negative achieved CR after additional irradiation up to the definitive dose, and the recurrence rate after CR was low in these patients. This indicates that a patient with negative biopsy at the time of 40 Gy has a good chance of complete cure after additional irradiation up to the definitive dose, regardless of neoadjuvant intent or definitive intent at the start of treatment. On the contrary, the rate of recurrence in patients with positive biopsy at the time of 40 Gy is considered high even if they achieved CR after further irradiation up to the definitive dose. This indicates that a patient with a positive biopsy at the time of 40 Gy has only a small chance of being cured by additional irradiation up to the definitive dose, and that perhaps surgery would be performed for such patients instead of further sessions of irradiation up to definitive dose. In fact, the present study showed that among patients with positive biopsies excluding patients with T1 tumors, there was a tendency of those who underwent neadjuvant CRT followed by surgery showing better survival than those with definitive CRT (31.8% vs 13.5%). However, in case of locally advanced tumors, if endoscopic biopsy at the time of 40 Gy is positive for malignancy, patients often do not achieve downstaging of those tumors and sometimes receive noncurative resection. Indication for surgery in such patients should be carefully considered by using diagnostic imaging. The usefulness of endoscopic biopsy in selecting treatment strategy after induction CRT needs further investigation.

In the present study, the incidences of positive biopsy after induction CRT in both the patients who received neoadjuvant CRT followed by surgery and those who received definitive CRT were relatively higher (nearly half of patients) than those reported in previous studies (range, 20%–30%).[27,32–35] One possible reason for the difference in the studies is that we performed endoscopic biopsy immediately, almost within 1 week after completion of 40 Gy irradiation, whereas endoscopic biopsy was often conducted some time after the completion of neoadjuvant CRT in the previous studies. Another possible reason is that the radiation dose at the time of endoscopic examination was relatively low in our study compared with those studies in which patients received more than 40 Gy irradiation (40–50.4 Gy) as neoadjuvant CRT, with the exception of the study by Schneider et al (36 Gy).[27]

In the present study, we performed endoscopic biopsy at the time of 40 Gy irradiation during the course of CRT, because the dose of irradiation used routinely in our hospital is 40 Gy for neoadjuvant CRT and mostly 60 Gy for definitive CRT. In previous studies of neoadjuvant CRT for locally advanced esophageal cancers, the total radiation dose varied with institutions and therapeutic regimens, ranging from 35 Gy to 50.4 Gy.[5–10,12–14] With regard to definitive CRT, a phase III randomized trial of combined-modality therapy for esophageal cancer, the RTOG 9405 (Intergroup 0123) study, which compared high-dose radiation therapy (64.8 Gy) with standard dose radiation (50.4 Gy), showed higher toxicity in the high-dose radiation group and no survival advantage.[42] Thus, the dose of 50.4 Gy has become the standard in definitive CRT in the United States. On the contrary, the current practice in Japan is to apply total radiation of more than 60 Gy in patients who undergo definitive CRT.[37,43] Although our results suggest that endoscopic biopsy at the time of 40 Gy irradiation may provide useful information for selection of therapy, the most appropriate time to obtain endoscopic biopsy to individualize therapy (surgery or completion of definitive CRT) may vary according to the dose of irradiation used in neoadjuvant and definitive CRT.

In conclusion, the present study demonstrated that endoscopic biopsy conducted at the time of 40 Gy irradiation predicts histopathological tumor regression and survival of patients who receive neoadjuvant CRT followed by surgery and it also predicts the clinical response and survival of patients who receive definitive CRT. Further studies are required to confirm the usefulness of endoscopic biopsy in the course of CRT for selection of treatment strategy based on response to induction CRT, surgery, or completion of CRT up to the definitive dose.

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Comparison of 4-Weekly vs 3-Weekly Gemcitabine as Adjuvant Chemotherapy Following Curative Resection for Biliary Tract Cancer: A Prospective Randomized Controlled Trial

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ABSTRACT

Background: *Surgery for biliary tract cancer, including pancreatoduodenectomy and major hepatectomy, is too aggressive and does not allow postoperative gemcitabine to be administered by the usual dosage protocol. We hypothesized that the feasibility of 3-weekly protocol (days 1 and 8, every 3 weeks) of adjuvant gemcitabine therapy may be superior to the usual 4-weekly protocol (days 1, 8, and 15 every 4 weeks).* ***Method***: *We compared the outcomes of 6 cycles of the 4-weekly protocol and 9 cycles of the 3-weekly protocol in a prospective randomized setting. The primary endpoint was the completion rate, and the secondary endpoints were the adverse events and the recurrence-free survival rate.* ***Results***: *Totally, 27 patients were enrolled. The protocol could be completed without any omittances and/or dose modifications in two patients (14%) of the 4-weekly protocol, and three patients (23%) of the 3-weekly protocol ($p = 0.8099$); grade 3/4 neutropenia occurred in almost all the remaining (70%) patients. The relative dose intensity was 72% in the 4-weekly protocol and 78% in the 3-weekly protocol. There was no significant difference in the recurrence-free survival rate.* ***Conclusion***: *The 3-weekly protocol did not yield superior completion, adverse events or recurrence-free survival rates as compared to the 4-week protocol.* ***Trial Registration***: *UMIN-CTR, UMIN000001020.*

Keywords: *Biliary Tract Cancer, Adjuvant Therapy, Gemcitabine*

1. Introduction

The prognosis of biliary tract cancer (BTC) is still poor [1-6]. Although surgical treatment remains the only potentially curative treatment, the overall 5-year survival rate remains approximately 40% [6,7]. This uncommon cancer is not yet well-studied because of the complexity of its classification and surgical procedures, andthe high perioperative morbidity, including liver dysfunction and cholangitis. Therefore, no (neo-) adjuvant chemotherapy has been established for these patients [8]. On the other hand, a pooled analysis [9] and multicenter retrospective analysis [10] revealed the potential efficacy of gemcitabine for unresectable and recurrent BTC, and a prospective randomized study revealed the survival benefit of gemcitabine-based chemotherapy in these patients [11,12]. Moreover, this drug has been shown to have a good safety profile, with a low incidence of grade 3/4 toxicities [13].

Based on this background, gemcitabine was introduced for adjuvant therapy after curative resection of BTC. However, it is difficult in the clinical setting to continue the usual4-weekly protocol (1000 mg/m^2 on days 1, 8

and 15 every 4 weeks) after BTC surgery. And, there have been several reports of gemcitabine-based adjuvant chemo therapy following major hepatic to mine for BTC or pancrea to duodenectomy for pancreatic cancer, which have suggested that dose modification is often necessary or that the usual 4-weekly protocol could not be, or was not applied [14-16]. Because of the morbidity, liver dysfunction, and low performance status (PS) after BTC surgery (major hepatectomy/pancreatoduodenectomy), it would seem difficult to complete the usual 4-weekly protocol, and frequent pauses during the adjuvant during therapy would be necessary.

For the above reasons, we hypothesized that postoperative gemcitabine therapy by the3-weekly protocol (1000 mg/m^2 on days 1 and 8 every 3 weeks) might be more feasible and superior to the 4-weekly protocol, because it would allow more treatment pauses. In this study, we compared the completion rate between patients assigned to the 4-weekly and 3-weekly protocols, for the same planned total dosage (6 cycles for the 4-weekly protocol and 9 cycles for the 3-weekly protocol). Because no feasibility studies of adjuvant gemcitabine therapy have been reported yet for BTC, we also comparatively estimated the frequency and severity of adverse events and the treatment efficacy (recurrence-free survival) between the two protocols. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR, UMIN 000001020) in JAPAN.

2. Patients and Methods

2.1. Study Design and Endpoints

We designed this open, multicenter, randomized controlled trial to explore the feasibility and efficacy of adjuvant gemcitabine therapy for BTC. The trial was initiated by the Osaka University Biliary Tract Cancer Treatment Group (OBCG, affiliated to the Multicenter Clinical Study Group of Osaka), Department of Surgery, Graduate School of Medicine, Osaka University. The protocol was approved by the institutional review board a teach hospital, and written informed consent was obtained from each of the patients.

The primary end point was the completion rate of adjuvant therapy. As control, we employed the 4-weekly protocol of gemcitabine treatment, which was studied in the CONKO-001 study after similar surgeries for pancreatic cancer [15].

The secondary end points included the frequency and severity of adverse events, for the purpose of collecting data on adverse events associated with adjuvant therapy, and the recurrence-free survival.

We determined that alpha and beta errors were 10% and 20%, respectively, to explore the feasibility and efficacy. After calculation of the sample seize, we determined that a total of 40 patients would be required. The study was started in August 2007 and completed in March 2010.

2.2. Patient Selection

Patients with histologically confirmed BTC (extrahepatic bile duct cancer, gall bladder cancer, or cancer of the papilla of Vater, UICC-stage II to IV [17]), who underwent macroscopic complete resection and no other therapy than surgery, were eligible for the study, and adjuvant therapy was to be started from 4 to 12 weeks after the surgery. Other eligibility criteria included age ≥20 years, Eastern Cooperative Oncology Group (ECOG) PS 0/1 [18], adequate hematological, liver and renal function (hemoglobin > 10 g/dl, leukocytes > 4000/μl, neutrophils > 2000/μl, platelets > 100,000/μl, serum transaminases < 4× the upper limit of normal (ULN), serum bilirubin < 2× ULN, andserum creatinine <ULN).

Patients were excluded if they had active interstitial pneumonia, severe edema, pregnancy, active infection, severe underlying disease (impaired cardiac function, active peptic ulcer, ileus, uncontrolled diabetes mellitus, etc.), severe allergy, severe mental disorders, or active another cancer.

2.3. Treatment and Dose Modification

Standard surgical procedures were used depending on BTC involvement. Eligible patients were randomly assigned by a computer-generated central randomization with stratification for institution and surgical procedure (pancrea to duodenectomy vs. others).

Patients assigned to the 4-weekly protocol received 6 cycles, with each cycle consisting of three weekly administrations of intravenous gemcitabine at 1000 mg/m^2, followed by a1-week drug-free pause. Patients assigned to the 3-weekly protocol received 9 cycles, each cycle-consisting of two weekly administrations of gemcitabine, followed by a1-week drug-free pause.

The first administration in each cycle was started with adequate hematological, liver, and renal functions (leukocytes > 3000/μl or neutrophils > 1500/μl, platelets > 100,000/μl, serum transaminases < 5 × ULN, serum bilirubin < 3 × ULN, and serum creatinine < ULN). When the first administration of any cycle could not be started within 28 days, the patient was withdrawn from the study.

For the second or third administrations in each cycle, the following were set in addition to first administration criteria; leukocytes > 2000/μl or neutrophils > 1000/μl, and platelet > 70,000/μl. When the above-mentioned criteriawere not fulfilled, dose modification was necessary for the next administration, as follows: 1000 mg/m^2

> 800 mg/m^2 > 600 mg/m^2 > withdrawal from the study. Omitted doses of gemcitabine were not replaced. During the study, neither anti-cancer therapies were allowed. Patients were withdrawn from the study for any of the following reason: disease recurrence, patient's desire to discontinue, or unacceptable treatment toxicity

2.4. Assessments

Prior to enrollment in the study, all patient sunder went routine examinations and laboratory studies. Tumor assessments were performed on the chest X-ray and abdominal computed tomo graphic or magnetic resonance images, prior to the adjuvant therapy and every 3 months.

During the study, vitalsigns, laboratory studies, PS, and toxicities/adverse events were evaluated prior to each administration. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE version 3.0).

The overall survival and recurrence-free survival rates were calculated by the Kaplan–Meiermethod, and the log-rank test was used for comparisons. Student's t-test or chi-square test was used to compare any differences. P-values <0.05 were considered to indicate statistical significance. All statistical analyses were performed using the StatView J-5.0 software (SAS, Cary, NC).

3. Results

3.1. Results

A total of 27 patients were recruited into the study from 13 centersinthe Kansai area, Japan. Recruitment was planned for ending in March 2010, and we analyzed the data at thistime to determine whether the study should be extended or not. There were no differences in the completion rate between the protocols, and we completed the study in 27 patients. The patients were randomized to the 4-weekly protocol (n = 14) and or the 3-weekly protocol (n = 13).The baseline characteristics of the eligible patients are shown in **Table 1**, and there were no statistically differences between the two protocols. All patients had adenocarcinoma, the majority had Stage IIB BTC, 15 (56%) underwent pancreatoduodenectomy, and 8 (30%) underwent major liver surgery. The median time from surgery to the start of chemotherapy was 62 days (24 - 86), with no significant difference between the protocols.

3.2. Treatment Delivery

The number of patients in whom the adjuvant therapy could be completed without any omittances and/or dose modifications was 2 (14%) in the 4-weekly protocol and 2 (15%) in the 3-weekly protocol (p = 0.8099, **Table 2**). The scheduled treatment cycles could not be completed in 3 patients of the 4-weekly protocol and in 4 patients of the 3-weekly protocol. The median number of administrations was16 for both protocols, and the median durations of administration were 168 days and 189 days, depending on the protocol bias. The median total dosages of adjuvant gemcitabine were 13,000 mg/m^2 in the 4-weekly protocol, and 14,000 mg/m^2 in the 3-weekly protocol. The median relative dose intensities were 72.2% and 77.8%, respectively. The potential need for omittance on account of grade 3 hematological or other events occurred first at the 3rd administration in the 4-weekly protocol and at the 10th administration in the 3-weekly protocol.

Table 1. Patient characteristics.

	4-Weekly Protocol	3-Weekly Protocol	p-value
Age	64 (56 - 76)*	68 (57 - 77)	0.6334
Sex (male/ female)	8/6	6/7	0.5680
Performance status (0/1)	11/3	10/3	0.9180
Body weight (kg)	52 (41 - 65)	54 (34 - 70)	0.9478
Primary lesion			0.3624
Hilar cancer	2	5	
Inferior bile duct cancer	4	4	
Gall bladder cancer	4	1	
Cancer of the Papilla of Vater	4	3	
Surgery			0.4649
Pancreatoduodenectomy	8	7	
Liver bed resection	3	1	
Hemihepatectomy or more	3	5	
UICC-Stage			0.3576
IIA	5	6	
IIB	7	7	
III	2	0	

*The number indicates the median (minimum-maximum).

In the subcategory analysis, the completion rates were 26.7% (4/15) and 9.0% (1/11) in pancreatoduodenectomy and other-surgery, respectively (p = 0.5691). The potential need for omittance occurred first at the 8th administration in pancreatoduodenectomy and at the 2nd administration in other-surgery. The dose intensities were 78% and 73%, respectively. In 4-weekly and 3-weekly protocol after pancreatoduodenectomy, the completion rates were 12.5% (1/8) and 42.9% (3/7), respectively (p = 0.5263). The potential need for omittance occurred first at the 3rd administration in the 4-weekly protocol, and at the 12th in the 3-weekly protocol (p = 0.0188). The dose intensities were 72% and 86%, respectively (p = 0.1152). After other-surgery, the completion rateswere 16.7% (1/6) and 0% (0/6), the potential need for omittance occurred first at the 3rd and 2nd administrations, and the dose intensities were 71% and 75%, respectively, in the 4-weekly and 3-weekly protocols, with no statistically significant differences between the two protocols.

3.3. Toxicity

Grade 4 toxicities were encountered only in the 4-weekly protocol (grade 4 neutropenia), while grade 3 neutropenia was noted at a high frequency (64% and 69%, respectively) in both protocols (Table 3). Grade 3/4 nonhematologic toxicity occurred only infrequently in either

Table 2. Completion rate, number of administrations, and total dose.

	4-Weekly Protocol	3-Weekly Protocol	p-value
n (Pancreatoduodenectomy / other surgery)	14 (8/6)	13 (7/6)	
Complete all cycles	2 (1/1) 14%	3 (3/0) 23%	0.8099
Adjuvant therapy completed with omittance and/ or dose modification	9 (5/4) 64%	6 (2/4) 46%	
Could not complete all cycles	2 (1/1) 14%	3 (1/1) 23%	
Recurrence during therapy	1 (1/0)	1 (0/1)	
Number of administrations	16	16	
Total dose (mg/m^2)	13000 (2600 - 18000)	14000 (2400 - 18000)	0.7017

Table 3. Number of patients with maximum grade of adverse events during the treatment.

		4-Weekly Protocol				3-Weekly Protocol			
		Gr1*	Gr2	Gr3	Gr4	Gr1	Gr2	Gr3	Gr4
Performance status		5				5	3	1	
Hematological	Leukocytes	1	8	2		1	8	2	
	Neutrophils		3	9	1		3	9	
	Hemoglobin	11	3			6	5	1	
	Platelets	8	3	1		3	2		
Laboratory	Aspartate aminotransferase	6	1	1		4	1		
	Alanine aminotransferase	4	1			4	1		
	Bilirubin		1			1			
	Creatinine								
Constitutional symptom	Fatigue	5	2			8	2		
	Fever	3	1			3	1		
Gastrointestinal	Nausea / Vomiting	3				5	1		
	Anorexia	2	2			2	3		
	Diarrhea	1				8			
	Stomatitis	2							
	Constipation	2							
Dermatology	Alopecia					1			
	Rash		1						
Edema							1		

*Grade 1.

protocol. A total of 27 serious adverse events (grade 3/4) were reported in the 21 patients (12 patients of the 4-weekly protocol and 9 patients of the 3-weekly protocol). In regard to the development of constitutional symptoms (PS and general fatigue) and gastrointestinal toxicity (nausea/vomiting), 52% and22% of patients developed grade 1/2 toxicities in the 4-weekly and 3-weekly protocolsandonly one patient experienced grade 3/4 toxicity (PS 3, just before withdrawal from the study).

3.4. Efficacy

The survival curves are shown in **Figure 1**. For a median follow-up of 17 months, the 1- and 2-year recurrence-free survival rates were 77% and 55% in the 4-weekly protocol, and 84% and 53% in the 3-weekly protocol, respectively (p = 0.8295). The 1- and 2-year overall survival rates were 100% and 75% in the 4-weekly protocol, and 100% and 71% in the 3-weekly protocol, respectively (p = 0.5876).

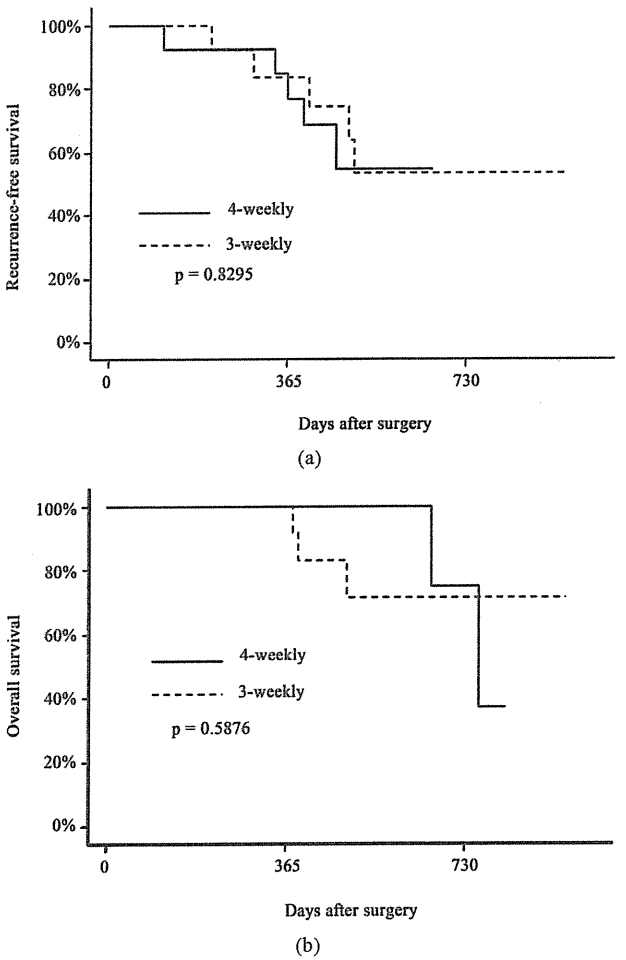

Figure 1. Recurrence-free survival (a) and overall survival (b) of biliary tract cancer patients after surgical resection. Solid line indicates the survival curve of the patients who received 6 cycles of adjuvant gemcitabine therapy by the 4-weekly protocol (1000 mg/m^2 on days 1, 8 and 15 every 4 weeks), and the dotted line indicates that of those who received 9 cycles of adjuvant therapy by the 3-weekly protocol (1000 mg/m^2 on days 1 and 8 every 3 weeks). There were no significant differences in either parameter between the two groups.

4. Discussion

There is as yet no feasibility-certified adjuvant therapy for BTC, especially when gemcitabine is used. In pancrea to duodenectomy for pancreatic cancer, the CONKO-001 and JSAP-02 trials revealed that dose modification was frequently necessary for completion [15,16]. In BTC, Murakami *et al.* presented gemcitabine-based adjuvant chemotherapy, however, all patients could not complete the protocol, even for biweekly 700 mg/m^2 gemcitabine-based chemotherapy [14]. Clinically, several omittances are necessary in the usual 4-weekly protocol of gemcitabine after BTC surgery, and it become like the 3-weekly protocol. Therefore, we hypothesized that the 3-weekly protocol may be more feasible and superior (higher completion rate). However, the 3-weekly protocol was not superior to the 4-weekly protocol in the completion rate, frequency of adverse events, or disease-free survival. In other words, the administration protocol did not influence the completion rate of adjuvant gemcitabine therapy for BTC.

In regard to the relative dose intensity, approximately 75% was achieved on average. In CONKO-001 and JSAP-02 (Note; including distal pancreatectomy), the relative dose intensity was approximately 90% [15,16]. In our data, the dose intensity was 78% in pancreatoduodenectomy, but only 73% in other-surgery (including liver resection). It would therefore seem that the dose intensity is related to the surgical stress. On the other hand, the dose intensity in pancrea to duodenectomy treated by the 3-weekly protocol was 86%, whereas that in the subgroup treated by the 4-weekly protocol was approximately 70%. Similar data were obtained for the first drug omittance. In pancrea to duodenectomy, the first potential omittance was necessitated much later in the 3-weekly protocol than in the 4-weekly protocol, the difference being statistically significant. In other-surgery, the dose intensity was only 70% in both protocols and the first omittances were necessitated at the 2nd - 3rd administrations; these data were inferior to the data in the 3-weekly subgroup of pancrea to duodenectomy. In addition, some patients who underwent hepatectomy received only few gemcitabine administrations before withdrawal. This led us to speculate that in pancrea to duodenectomy, the 3-weekly protocol might be better, but that in more

aggressive surgery, like major liver resection, neither the 4-weekly nor the 3-weekly protocol might be feasible for adjuvant therapy. This study might not have enough power to mention these speculations. It would be necessary to perform phase I study to yield a higher dose intensity, followed by phase II/III study in a larger study group.

In relation to treatment toxicity, we encountered a high frequency of grade 3/4 neutropenia, and also of grade 2 constitutional symptoms and gastrointestinal adverse events. Although there is little information about neutropenia in previous reports [14,15], the JSAP-02 reported a high frequency of grade 3/4 neutropenia [16], similar to our study. This adjuvant therapy seemed to yield a high frequency of not grade 3/4 leukopenia, but of grade 3/4 neutropenia. After surgery for BTC, the patients sometimes develop cholangitis, and with the occurrence of severe neutropenia, liver abscess and/or sepsis could occur. Patients must therefore be closely monitored for the development of neutropenia. In regard to constitutional symptoms and gastrointestinal adverse events, approximately 25% of the patients with such adverse events were unable to carry on with their work activities or needed drip infusions (grade 2 toxicity), suggesting that treatment of these patients in the outpatient setting might be difficult.

In regard to the efficacy, we compared our historical data [4-6] and the report from the Japanese Society of Biliary Surgery (JSBS) [7,19], and data on gemcitabine-based adjuvant therapy by Murakami et al. [14]. In regard to the recurrence-free survival, the data in our present study (77% - 84% at 1 year and 52% - 55% at 2 years) were similar to those reported by Murakami et al. (recurrence-free survival: 79% at 1 year and 60% at 3 years) [14]. In terms of overall survival, the rate in our study 75% - 71% at 2 years, as compared to historical data (without adjuvant therapy) of approximately 65% at 2 years and 60% - 63% at 3 years. The JSBS reported an overall survival rate of 40% - 65% at 3 years. Based on the above findings, we suggest that there remains the possibility of a survival benefit of adjuvant gemcitabine therapy after BTC surgery.

In conclusion, the 3-weekly gemcitabine treatment protocol was not superior to the 4-weekly protocol in terms of the completion rate, relative dose intensity, adverse events or recurrence-free survival, among patients receiving adjuvant therapy following BTC surgery; a high frequency of grade 3/4 neutropenia was found in both the protocols. Furthermore, the treatment could be completed without any interruptions and/or dose modifications in only approximately 10% of the patients. Our findings suggest the possibility of the dose intensity depending on the aggressiveness level of the surgical procedures, and further investigation is warranted. For a precise evaluation of the efficacy in a feasibility study for adjuvant therapy after aggressive BTC surgery, a prospective randomized study with a large number of patients would be necessary.

5. Disclosure

The authors have no conflict of interests to declare.

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Multicenter Phase I/II Study of Docetaxel, Cisplatin and Fluorouracil Combination Chemotherapy in Patients with Advanced or Recurrent Squamous Cell Carcinoma of the Esophagus

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Key Words
Chemotherapy · Cisplatin · Docetaxel · Fluorouracil · Squamous cell carcinoma of esophagus

Abstract
Objective: Esophageal squamous cell carcinoma (ESCC) is refractory to current therapeutic regimens and more effective therapies are imperative. To this end, we conducted a multicenter phase I/II trial of docetaxel, cisplatin, and fluorouracil (DCF) combination chemotherapy for ESCC. ***Methods:*** The study subjects were 46 patients with advanced or recurrent ESCC. Treatment included docetaxel at 60, 70, and 75 mg/m^2, cisplatin at 70 mg/m^2 on day 1, and daily fluorouracil at 700 mg/m^2 on days 1 through 5. The recommended dose of docetaxel was determined in phase I, while the response rate (RR) and progression-free survival rates were analyzed in phase II. ***Results:*** The recommended dose was determined to be 70 mg/m^2 in phase I. In phase II, the RR was 72.5%. Interim analysis showed median and 1-year progression-free survival of 14 months and 55.6%, respectively. Grade 3/4 toxicities of leukopenia and neutropenia occurred in 72.5 and 90% of patients, respectively. No treatment-related death was recorded. Surgical resection was subsequently performed in 20 patients after chemotherapy, and curative resection was achieved in 19. ***Conclusion:*** DCF was tolerable and effective for advanced and recurrent ESCC. Such findings might encourage a change in the treatment strategy for ESCC.

Introduction

Advanced esophageal squamous cell carcinoma (ESCC) is one of the most refractory cancers and is associated with poor outcome. Systemic chemotherapy is regarded as one of the most effective treatments for ESCC, and currently forms an important part of the multidisciplinary treatment approach for advanced and metastatic disease. In particular, fluorouracil and cisplatin (FP) combination therapy has become a standard choice. FP regimens result in partial response rates of 25–35% [1, 2], with a 1-year survival rate ranging from 27.8 to 37.6% [3–5], and median survival time of 9.2 months for responders and 5.3 months for nonresponders. To improve the prognosis of patients with advanced ESCC, more effective regimens are urgently needed.

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Docetaxel (DTX) is a relatively new cytotoxic antineoplastic agent currently used for the treatment of various cancers. Many studies have reported that taxanes have significant activity in patients with advanced and metastatic esophageal carcinoma [6–10]. As a single agent, DTX is reported to have achieved a partial response rate (RR) of 20.4% in patients with metastatic esophageal cancer, raising hope that this agent could be useful in esophageal cancer [6]. Several studies of head and neck cancer and gastric cancer further indicated that the regimen of DTX plus cisplatin and fluorouracil (DCF) produced a higher RR and improved overall survival than the conventional FP regimen [11–14]. However, there are only a few reports on the efficacy and feasibility of DCF for esophageal cancer, especially ESCC. To evaluate the safety and efficacy of DCF, we conducted a multicenter phase I/II trial for ESCC.

Patients and Methods

Eligibility Criteria

Patients were regarded as eligible if they had histopathologically confirmed ESCC. The selection criteria were as follows: (1) clinically confirmed T4 and/or M1 tumors including nonregional lymph node metastases according to the 6th edition of the Tumor-Node-Metastasis Classification of the International Union Against Cancer (UICC) or recurrent tumor after esophagectomy; (2) assessable lesions according to the criteria of the Japanese Society for Esophageal Diseases; (3) no prior treatment for ESCC in primary cases and no prior chemotherapy and/or radiotherapy for ESCC in recurrent cases; (4) age 20–75 years; (5) Eastern Cooperative Oncology Group (ECOG) performance status within 0–1; (6) normal function of the major organs (indicated by leukocyte count >4.0 × 10^9/l, platelet count >100 × 10^9/l, hemoglobin >9.0 g/dl, serum AST/ALT less than 2× upper limit of normal, serum bilirubin <1.5 mg/dl, and calculated creatinine clearance >60 ml/min); (7) a life expectancy of at least 3 months, (8) lack of serious complications such as heart disease, pulmonary fibrosis, interstitial pneumonia, and bleeding tendency; (9) lack of overt infection (fever above 38°C); (10) lack of active double primary cancer; (11) lack of symptoms related to peripheral nerve lesions or edema of more than grade 2 according to the Common Terminology Criteria for Adverse Events of the National Cancer Institute (NCI-CTCAE, version 3); (12) negative history of drug hypersensitivity; (13) absence of current or previous brain metastases, and (14) absence of pregnancy, breast-feeding, or consideration of pregnancy.

All patients provided written informed consent before enrollment. The study protocol was approved by the instruction review board of each participating hospital.

Pretreatment Evaluation

Before treatment, the patients underwent enhanced computed tomography (CT) of the chest and abdomen, esophagogastroscopy, and positron emission tomography to assess the pretreatment tumor stage. Positive lymph nodes were defined as those with a diameter of at least 1 cm on CT scans [15].

Treatment Plan

We conducted a brief phase I trial to determine the maximum tolerated dose (MTD) and recommended dose (RD), followed by a larger phase II study using the regimen determined by the phase I trial to evaluate the response rate, survival, and toxicities of the DCF treatment. The following supportive therapy was routinely used in this study: patients received sufficient hydration and premedication consisting of dexamethasone infusion plus administration of cimetidine 1 day before infusion of DTX. Furthermore, antiemetics and 5-hydroxytryptamine-3 antagonist were routinely administered twice a day on days 1–3 with adjustment of dose and period as needed. No prophylactic granulocyte colony-stimulating factor (GCSF) or antibiotics were provided. However, GCSF was administered subcutaneously when febrile neutropenia or grade 4 neutropenia was encountered, and the use of GCSF in such cases was continued until the leukocyte count increased to more than 5,000 cells. GCSF was also administered upon the development of grade 3 neutropenia in patients who required GCSF in previous cycles.

Phase I Study. The dose of DTX was escalated using the following protocol: level 1: 60 mg/m^2, level 2: 70 mg/m^2, and level 3: 75 mg/m^2, intravenously infused over 60–120 min on day 1. Cisplatin and fluorouracil were administered at a dose of 70 mg/m^2 infused over 120 min on day 1 and 700 mg/m^2 continuously infused from day 1 to day 5, respectively. The above course was repeated every 21 days for up to two cycles. The dose-limiting toxicities (DLTs) during chemotherapy were defined as follows: (1) grade 4 leukopenia or neutropenia persisting for ≥5 days; (2) grade 3 or higher neutropenia with fever (febrile neutropenia); (3) grade 4 thrombocytopenia; (4) grade 3 or higher nonhematological toxicity except for anorexia, vomiting, nausea, constipation, fatigue, stomatitis, electrolyte abnormality, and diarrhea that resolved after 3 days, and (5) the need to delay therapy for more than 14 days because of toxicity. The MTD was defined as follows: if none of the 3 patients at level 1 developed DLTs, the dose was escalated to the next level. If 1 of the 3 patients had DLTs, 3 additional patients received the dose at the same level. If 2 or fewer patients showed DLTs, the dose was escalated to the next level. If 3 or more patients showed DLTs, that dose was defined as the MTD.

We defined the dose below the MTD as the RD, if the DCF chemotherapy in this dose was tolerable, and this regimen would be advanced into the phase II study. If the MTD was the level 1 dose, this trial was stopped and the phase II study would not proceed.

Phase II Study. After completion of the phase I study, the phase II study started at the RD defined by the phase I study. The treatment regimen was repeated every 3 weeks for up to two cycles according to the protocol, unless progression, patient's refusal, or unacceptable toxicity occurred. The next course commenced when the patient maintained particular biological parameters to meet the following criteria: (1) <grade 1 leukopenia, neutropenia, or thrombocytopenia; (2) urea nitrogen, creatinine, alanine aminotransferase, and aspartate aminotransferase remain within twice the normal limits, and (3) performance status within 0–1. If these criteria were not satisfied for more than 6 weeks after day 1 of the first cycle of chemotherapy, the patient was withdrawn from the study. The subsequent treatment was not defined.

Table 1. Patient characteristics

	Phase I	Phase II
Number	9	40
Median age (range), years	65 (53–71)	64 (43–75)
Sex (male:female)	9:0	34: 6
ECOG performance status (0:1)	6:3	29:11
Disease status (initial:recurrent)	9:0	39:1
Location of primary tumor (Ut:Mt:Lt)	1:4:4	10:18:11
Depth of invasion of primary tumor (T2:T3:T4)	0:4:5	4:13:22
Metastatic organ		
Nonregional LN	5	15
Liver	0	2
Lung	1	3
Adrenal	1	0
Cancer stage (III:IV)	2:7	18:21
Location of recurrent tumor		regional LN

Ut = Upper thoracic; Mt = middle thoracic; Lt = lower thoracic; LN = lymph nodes.

Evaluation

Toxicity was evaluated according to NCI-CTCAE, version 3. Clinical tumor responses were evaluated by esophagoscopy and CT after every cycle of chemotherapy. The responses of primary tumors and metastatic tumors in lymph nodes and distant organs to chemotherapy were evaluated according to the criteria of the Japanese Society for Esophageal Disease [16]. The primary tumor was defined as a measurable lesion and the response was evaluated by CT scan when the longest diameter of primary tumor was at least 20 mm. The response of primary tumor was evaluated by esophagoscopy when there was no measurable lesion. Partial response was defined as ≥50% decrease in the products of the two perpendicular diameters (TPD) of measurable lesions or marked morphological improvements (i.e., tumor regression, flattening of a raised ulcer margin, and shallowing and clearing of ulcer base) confirmed by esophagoscopy. Stable disease was defined as <50% reduction and <25% increase in the TPD of a measurable lesion, or a slight decrease or no change in tumor size confirmed by esophagoscopy. Progressive disease was defined as ≥25% increase in the TPD of measurable lesions, enlargement of the tumor, and/or the appearance of new lesions confirmed by esophagoscopy. Complete response was defined as the complete disappearance of all evidence of tumor, including negative biopsy results. The final response was assigned based on the evaluation after two cycles of the combination chemotherapy. If the treatment was not repeated past two cycles because of tumor progression, due to the patient's refusal or an unacceptable outcome, the assignment after two cycles was defined as the clinical tumor response.

In patients who underwent surgery, the primary tumors were examined for histopathological changes, which were classified as grade 3 (markedly effective; no viable cancer cells), grade 2 (moderately effective; 'viable' cancer cells account for less than 1/3 of tumor tissue, while other cancer cells showed severe degeneration or necrosis), and grade 1 (slightly effective, where apparently 'viable' cancer cells accounted for 1/3 or more of the tumor tissue, but there was some evidence of degenerating cancer tissue or cells). Grade 1 lesions were also subclassified into grade 1a (viable cancer cells accounted for 2/3 or more of tumor tissue), grade 1b (viable cancer cells accounted for ≥1/3, but <2/3, of tumor tissue), and grade 0 (ineffective, denoting no discernible therapeutic effect on cancer tissue or cells).

Statistical Analysis

The primary objective of the phase I study was to determine the MTD and RD. In phase II study, the primary objective was to assess the objective response rate (ORR) using the defined RD, and the secondary objective was to evaluate overall survival and treatment-related toxicity. The sample size was assumed based on findings that the ORR with previous FP therapies ranged from 33 to 36%, and we hypothesized that this regimen (DCF) would achieve an ORR of 55%. The phase II study enrolled 38 patients, a number that was required to confirm the null hypothesis that 95% confidence interval of the expected ORR (55%) would be less than 35% under conditions of an α error of 0.05 and a β error of 0.2.

Results

Patient Characteristics

Between October 2008 and June 2010, the phase I study enrolled 9 patients followed by the enrollment of 40 patients in the phase II study. Of the total number, 86, 15 and 9% received treatment at Osaka University Hospital, Osaka Medical Center for Cancer and Cardiovascular Disease, and Kinki University Hospital, respectively. The baseline characteristics of all patients are listed in table 1. In the phase I study, the median age was 65 years (53–71), all enrolled patients were males, and they were all new cases. The location of the primary tumor was the upper esophagus in 1, middle in 4, and lower thoracic esophagus in 4. Two and 7 patients had stage III and IV disease, respectively, at enrollment. In the phase II study, the median age was 64 years (43–75) and 34 of 40 patients were males. Of the 40 patients, 39 were new cases and 1 patient had recurrent tumor in the cervical paraesophageal lymph nodes. The location of the primary tumor in patients of the phase II study was the upper esophagus in 10, the middle esophagus in 18, and the lower thoracic esophagus in 11. At enrollment, 18, 21, and 1 patients had stage III disease, IV disease, and recurrence, respectively.

Phase I Study

Hematological and nonhematological toxicities encountered in the phase I study are summarized in table 2. At level 1, 2 patients had grade 3 leukopenia and neutro-

Table 2. Toxicities recorded in phase I trial

	NCI-CTC grade				Grades 3/4 %
	1	2	3	4	
Hematological toxicities					
Level 1 (n = 3)					
Leukopenia	0	1	2	0	66.7
Neutropenia	0	1	2	0	66.7
Anemia	0	0	1	0	33.3
Thrombocytopenia	0	0	0	0	0
Level 2 (n = 3)					
Leukopenia	1	1	0	1	33.3
Neutropenia	0	0	2	1	100
Anemia	1	0	0	0	0
Thrombocytopenia	0	0	0	0	0
Level 3 (n = 3)					
Leukopenia	0	0	0	3	100
Neutropenia	0	0	0	3	100
Anemia	1	0	1	0	33.3
Thrombocytopenia	1	0	0	0	0
Nonhematological toxicities					
Level 1 (n = 3)					
Anorexia	0	2	0	0	0
Nausea	0	3	0	0	0
Diarrhea	0	0	1	0	33.3
Stomatitis	0	1	1	0	33.3
Febrile neutropenia	0	0	0	0	0
Level 2 (n = 3)					
Anorexia	0	2	0	0	0
Nausea	1	0	0	0	0
Diarrhea	0	0	0	0	0
Stomatitis	0	2	0	0	0
Febrile neutropenia	0	0	0	0	0
Level 3 (n = 3)					
Anorexia	0	2	0	0	0
Nausea	0	1	0	0	0
Diarrhea	0	0	1	0	33.3
Stomatitis	1	1	0	0	0
Febrile neutropenia	0	0	1	0	1

Data represent number of patients. NCI-CTC = National Cancer Institute Common Toxicity Criteria.

Table 3. Toxicities recorded in phase II trial

	NCI-CTC grade				Grades 3/4 %
	1	2	3	4	
Hematological toxicities					
Leukopenia	1	7	18	11	72.5
Neutropenia	1	1	11	25	90.0
Anemia	4	7	0	0	0
Thrombocytopenia	1	3	1	1	5.0
Nonhematological toxicities					
Anorexia	2	13	9	0	22.5
Nausea	4	3	5	0	12.5
Diarrhea	1	6	4	1	12.5
Stomatitis	3	9	2	0	5.0
Fatigue	2	2	3	0	4.5
Dementia	0	0	1	0	2.5
Venous thrombus	0	0	1	0	2.5
Febrile neutropenia	0	0	4	0	10.0

Data represent number of patients. NCI-CTC = National Cancer Institute Common Toxicity Criteria.

penia, and 1 patient had grade 3 anemia, while 1 patient developed grade 3 diarrhea and stomatitis as nonhematological toxicities, which did not meet the criteria of DLT. At level 2, 2 patients developed grade 3 neutropenia, and 1 patient had grade 4 leukopenia and neutropenia, which did not meet the criteria of DLT because recovery occurred within 3 days. No patient showed grade ≥3 nonhematological toxicity. At level 3, 3 patients developed grade 4 leukopenia and neutropenia, and 1 of these developed grade 3 febrile neutropenia. One patient developed grade 3 diarrhea persisting for more than 3 days. Consequently, we defined level 3 as the MTD and level 2 as RD because 2 of the latter patients met the criteria of DLT.

Phase II Study

Based on the phase I study, level 2 was determined as the RD that was used to treat the subsequent 37 patients in the phase II study, with the 3 patients designated as level 2 in the phase I study making up the total of 40 patients evaluated in the phase II study.

Hematological and nonhematological toxicities in the phase II study are summarized in table 3. Grade 3/4 leukopenia, neutropenia, and thrombocytopenia were observed in 29 (72.5%), 36 (90%), and 2 (5%) patients, respectively. The nadir of myelosuppression such as leukopenia and neutropenia was registered at days 8–10 after treatment in 30 (83%) of 36 patients, and continued for a median (minimum to maximum) of 3 (1–5) days. Febrile neutropenia of grade 3 occurred in 4 patients (10%), with anorexia and diarrhea in 9 and 5 patients, respectively, and grade 3 venous thrombus associated with percutaneous central venous catheter was reported in 1 patient. No death associated with the toxic effects occurred in the phase I or II study.

Of these, 33 (82.5%) patients completed the chemotherapy as defined by the protocol. The other 7 patients did not receive the second cycle of chemotherapy be-

Table 4. ORR for the recommended dose

	Primary tumor (n = 39)	Lymph nodes (n = 35)	Organ metastases (n = 5)	Overall (n = 40)
Complete response	7 (17.9%)	8 (22.9%)	0 (0%)	4 (10%)
Partial response	24 (61.5%)	17 (48.6%)	5 (100%)	25 (62.5%)
Stable disease	8 (20.5%)	9 (25.7%)	0 (0%)	10 (25%)
Progressive disease	0 (0%)	1 (2.9%)	0 (0%)	1 (2.5%)

Data represent number of patients with the percentage in parentheses.

Table 5. Subsequent treatments

Treatment modality	Number
Surgery	20
Chemotherapy	6
Chemoradiotherapy	6
Chemoradiotherapy and surgery	7
Best supportive care	1

cause of the chemotherapy as defined by the protocol: patient refusal by 2, grade 3 dementia in 1, grade 2 renal dysfunction in 1, and stable or progressive disease in 3 patients. The median number of treatment cycles was 2 cycles (range 1–6). The ORR of primary tumors was 79.4% (31/39 patients), the ORR of lymph nodes was 71.5% (25/35 patients), and the overall RR was 72.5% (29/40 patients) (table 4). Four patients showed a complete response (10%), 25 partial responses (62.5%), 10 patients stable disease (25%), and 1 progressive disease (2.5%).

Of the 40 patients in the phase II study, 39 (97.5%) received one or more subsequent treatments (table 5), while 1 patient remained untreated at his request. Six patients with organ metastases and/or extensive lymph node metastases, such as axillary or abdominal para-aortic nodes, received continuous DCF chemotherapy. Chemoradiotherapy was performed in 13 patients who were diagnosed as T4 in the postchemotherapy evaluation. Surgical resection was performed in 20 patients and all were considered to have achieved curative resection by postchemotherapy evaluation. Subtotal esophagectomy via right thoracotomy with two- or three-field lymphadenectomy was performed in 20 patients, of whom 19 received a curative resection (R0) and only 1 patient underwent a noncurative resection (R1). Postoperative complications (grade 2 or more according to NCI-CTCAE, version 3) occurred in only 1 of 20 patients in the form of grade 2 vocal cord palsy. There was no postoperative death. The histological effects in the primary tumors or recurrent lesions were grade 3 in 5 patients, grade 2 in 3, grade 1b in 5, and grade 1a in 7. These results also showed that 6 of 16 patients who were clinically diagnosed as positive for lymph node metastasis were pathologically node-negative.

We performed interim analysis with a median follow-up period of 12.4 months. The minimum follow-up period in surviving patients was 6.5 months. The median survival time was not reached, but the 1-year survival rate was 74.6%, the median progression-free survival (PFS) was 14 months, and the 1-year PFS was 55.6%.

Discussion

The present study was designed to evaluate the safety and efficacy of DTX, cisplatin, and fluorouracil used in combination (DCF) for T4 and/or metastatic ESCC. The overall ORR in this study was 72.5%, which was nearly twice that reported previously using the FP regimen, and about 1.5 times that of RRs reported using the FAP regimen [1, 17, 18]. These results highlight DCF as a promising successor of first-line chemotherapy for T4 and/or metastatic ESCC.

Interestingly, our results showed that there was no progression of disease in primary tumors when the disease control rate (DCR) was 100%. In this study, 22 of 40 patients (55%) were clinically diagnosed with invasion of adjacent organs (cT4): trachea or left main bronchus in 17 patients, aorta in 6 patients, and liver in 1 patient. These patients often underwent chemoradiotherapy as the first-line therapy because it was considered superior to chemotherapy in achieving local control. Previous studies indi-

cated that chemoradiotherapy resulted in RRs of 68–76% and DCRs of 88–100% [19–21]. The DCF regimen used herein for the patients with T4 disease resulted in 82% RR and a DCR of 100%. This indicates that the DCF chemotherapy was functionally active and that this approach might lead to more curative cases of unresectable advanced esophageal cancer. Recently, Takahashi et al. [22] used the DCF combination chemotherapy for metastatic ESCC and reported satisfactory tolerance and outcome. The dosage of DTX used in their study was 50 mg/m^2, which was relatively small compared with the 70 mg/m^2 dose used in our study. The difference in the dose between the two studies is probably related to differences in the rank of this regimen in the treatment strategy for advanced ESCC between their and our trial. Their study focused on the effectiveness and continuity of DCF, similar to standard phase I/II trials. On the other hand, our study was designed not only to determine the effectiveness but also usefulness of DCF as a first-line treatment regimen that would be applied in neoadjuvant or induction setting. Therefore, we set the dosage similar to that used by Vermorken et al. [11], which was fixed at 75 mg/m^2 as induction chemotherapy, rather than at that used by Takahashi et al. [22]. Our results showed that our regimen was tolerated well and was effective enough to be applied in neoadjuvant or induction therapy, although our regimen was highly toxic compared to that used by Takahashi et al. [22].

One limitation of this study was the assignment of the final response on evaluation conducted after two cycles of DCF, rather than evaluation at a subsequent time point. In nonrandomized trials where the response is the primary endpoint, the overall response is generally confirmed by evaluation at a subsequent time point [23]. Bogaerts et al. [24] indicated that removing the requirement for response confirmation led to a significant increase in the number of patients classified as responders, resulting in a relative increase in the response rate.

However, at the risk of overestimating the response rate, we developed a protocol in which the therapeutic regimen was repeated for up to two cycles and the subsequent treatment was not defined. This approach was selected because we focused on the application of DCF therapy as a first-line treatment in a multidisciplinary approach for advanced ESCC as well as the evaluation of the toxicity and response rates. Indeed, 12 patients with cT4 underwent standard surgery used for clinically resectable tumors, when they achieved downstaging after two cycles of DCF (i.e., from cT4 to <ycT3). On the other hand, 10 patients with ycT4 who did not achieve downstaging after two cycles of DCF received chemoradiotherapy as a secondary treatment.

Curative resection was subsequently achieved in 95% of these patients. There were no differences in perioperative outcome (e.g., operation time, blood loss, and complications) between the present and our previous study [25]. Therefore, it seems that surgical resection after DCF is safe and feasible. DCF might also be suitable as neoadjuvant treatment for patients scheduled for resection surgery. Analyses of data of cT4 patients showed promising results with 1-year overall survival and PFS rates of 72.7 and 54.5%, respectively. These results are encouraging regarding the potential clinical impact on future treatment strategies for ESCC.

The DCF regimen described herein produced side effects with high frequency, especially leukopenia and neutropenia, as reported previously [11–14]. However, it should be noted that the myelosuppression improved rapidly after GCSF administration and did not necessarily cause treatment delays. Posner et al. [12] also reported that although grade 3 or 4 neutropenia occurred in 83% of patients in the DCF group and in 56% of patients in the FP group, prolonged neutropenia was responsible for treatment-associated delays in only 1% of patients of the DCF group and 39% of patients of the FP group [12]. The frequency of nonhematological toxicities such as anorexia, nausea, and stomatitis noted in our protocol was similar to that reported in other studies [11–14] and caused no treatment delays. Although febrile neutropenia occurred in 10.8% of patients, these patients improved within 5 days and there were no treatment-related deaths. Thus, careful monitoring for adverse events, especially myelosuppression, should ensure safe completion of the DCF regimen.

We did not report the long-term outcome in the present study because the minimum (median) follow-up time for surviving patients was 6.5 (12.4) months. Interim analysis provided promising results with a median PFS of 14 months, which is more than twice longer than that of patients treated by the FP regimen [3–5]. Follow-up studies are needed to assess the long-term prognosis.

In conclusion, DCF was tolerable and useful for advanced and recurrent ESCC. This regimen could enhance the treatment strategy for not only unresectable and inoperable ESCC, but also resectable ESCC, although larger prospective studies are required.

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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Tyrosine Kinase Inhibitor PTK/ZK Enhances the Antitumor Effects of Interferon-α/5-Fluorouracil Therapy for Hepatocellular Carcinoma Cells

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ABSTRACT

Purpose. There is no standardized treatment for hepatocellular carcinoma (HCC) with portal vein tumor thrombus. We previously reported the efficacy of interferon-alpha and 5-fluorouracil combination (IFN/5-FU) therapy for these patients and the potential mechanism via the regulation of vascular endothelial growth factor (VEGF). In this study, we showed the VEGF-related effects of IFN/5-FU therapy using VEGF-receptor (VEGFR) selective inhibitor, PTK787/ZK222584 (PTK/ZK), in HCC cells.

Methods. Using two VEGF secreting and VEGFR expressing human HCC cell lines, PLC/PRF/5 and HuH7, we performed growth inhibitory assays in vitro and in vivo, apoptosis assay, cell cycle analysis, and Western blot analysis for the mechanism, with or without PTK/ZK in IFN/5-FU therapy.

Results. The combination of PTK/ZK and IFN/5-FU significantly inhibited cell growth in vitro and tended to reduce tumor growth in vivo in a HuH7 xenograft model in nude mice—in both cases without affecting VEGF secretion. PTK/ZK enhanced the IFN/5-FU induced apoptosis, based on increased proteins levels of Bax and reduced Bcl-xL and Bcl-2. Cell cycle analysis showed different results between the HCC cell lines following the combination therapy, possibly due to differences in p21 protein.

Conclusions. VEGF signaling inhibition would support an antitumor effect of IFN/5-FU therapy against HCC cell lines via induction of apoptosis and cell cycle delay.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Recent advances in surgical resection and liver transplantation have improved the local control of HCC; however, there is no standardized treatment for locally advanced HCC, defined by the presence of portal vein tumor thrombus. The prognosis for such patients remains extremely poor with surgery alone, and the reported median survival times are 6–14 months.[1–3] We recently started administering interferon-alpha (IFN-α) and 5-fluorouracil (5-FU) combination (IFN/5-FU) therapy as an adjuvant in cases of advanced HCC, with good clinical efficacy (1-survival rate: 100% vs. 41% in patients without IFN/5-FU historical controls).[4–7] One of our previous biochemical analyses revealed that this therapy controls tumor-associated angiogenesis by regulating endothelial growth factor (VEGF).[8,9] However, VEGF inhibition in this therapy is limited to only 30–40% and control of VEGF may contribute more tumor suppression under the therapy.[8,9]

On the other hand, recent advances in drug development targets angiogenic factors, in the field of HCC, because of its hypervascularity.[10,11] Among these factors, VEGF plays a central role, and VEGF-targeted agents have some clinical benefits for HCC, via tyrosine kinase blocking of VEGF-receptors (VEGFRs; Flt-1, Flk-1/KDR, and Flt-4).[12–16] We thus hypothesized that VEGF inhibition would enhance the VEGF-related antitumor effects of IFN/5-FU therapy. Actually, IFN/5-FU therapy partially inhibits VEGF secretion; therefore, the purpose of this study was

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evaluation for an additional benefit under VEGF inhibition in the IFN/5-FU therapy. In this study, we used this VEGFR selective inhibitor, PTK787/ZK22584 (PTK/ZK, Vatalanib®), rather than sorafenib, which is commonly used but targets several tyrosine kinases.[15,16] PTK/ZK is a selective potent inhibitor of all known VEGFR tyrosine kinases, particularly potent against Flt-1 and Flk-1/KDR, and the efficacy against human HCC cell lines in vitro and in vivo was reported.[17] Our results showed that PTK/ZK enhanced the direct effect of IFN/5-FU therapy on human HCC cells both in vitro and in vivo. Furthermore, we investigated the possible additional effects of such therapy on apoptosis and cell cycle, previously reported as the main mechanisms of IFN/5-FU.[18]

MATERIALS AND METHODS

Reagents and Cell Lines

Purified human IFN-α was obtained from Otsuka Pharmaceutical Co. (Tokyo, Japan), 5-FU was obtained from Kyowa Hakko Kirin Co. (Tokyo), and PTK/ZK was obtained from Bayer Schering Pharma (Berlin, Germany). The two human HCC cells lines, PLC/PRF/5 and HuH7, expressing IFN receptor type 2 (IFNAR2), were purchased from the Japanese Cancer Research Resources Bank.[18] Both lines were maintained as adherent monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mixture at 37°C in a humidified incubator with 5% CO_2 in air.

Growth Inhibitory Assays using PTK/ZK Combined with IFN/5-FU Therapy

The growth inhibitory effects were tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described previously.[18] The cells were incubated in medium with or without PTK/ZK (5 or 10 μM) and/or IFN (0.5 μg/ml)/5-FU (500 U/ml) for 96 hours, based on previous reports.[17,18] These assays were repeated at least three times, with similar results obtained. The proportion of MTT-positive cells incubated without drugs was denoted as 100% viability.

Flow Cytometric Analysis of Annexin V-FITC Binding

Apoptosis was measured based on the binding of FITC-conjugated annexin, as described previously.[19] Briefly, after treatment with IFN-α and 5-FU with or without various concentrations of PTK/ZK, the cultured cells (1×10^6) were incubated with binding buffer (10 mM HEPES, 140 mM NaCl, and 2.5 mM $CaCl_2$, pH 7.4) containing saturating concentrations of annexin V-FITC (BioVision, Mountain View, CA) and propidium iodide (PI) for 15 minutes at room temperature. After incubation, the cells were pelleted and analyzed by flow cytometry on a FACSCalibur (Becton Dickinson Immunocytometry Systems, BD, San Jose, CA), and data were processed using Cell Quest_software (BD).

Cell Cycle Analysis

Flow cytometric analysis was performed to assess the cell cycle, as described previously.[18] Briefly, after treatment with IFN-α and 5-FU with or without PTK/ZK (10 μM), cells were washed twice with phosphate-buffered saline (PBS) and then fixed in 70% cold ethanol for 4 hours before being washed and resuspended in 1 ml of PBS. PI (50 ml of 1 mg/ml solution in PBS) and RNase were added for 30 minutes at 37°C, and data were acquired on the FACSCalibur. Analysis of the cell cycle was performed using ModFIT software (BD).

Concentration of VEGF in Cell Culture Supernatants

After treatment with IFN-α and 5-FU with or without PTK/ZK (15 μM) for 48 hours, this conditioned medium was collected, and VEGF levels were analyzed using the human VEGF enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Camarillo, CA) as recommended by the manufacturer, as described previously.[8]

Western Blot Analysis

Cells were washed twice with ice-cold PBS and harvested from the culture dish. After centrifugation, the cell pellets were resuspended and lysed in RIPA buffer [25 mM Tris (pH 7.5), 50 mM NaCl, 0.5% sodium deoxycholate, 2% Nonidet P-40, 0.2% sodium dodecyl sulfate, 1 mM phenylmethylsulphonyl fluoride, and 500 KIE/ml aprotinin] containing phosphatase inhibitor. The extracts were centrifuged and the supernatant fractions were collected for Western blot analysis, performed as described previously.[20] The antibodies were used at 1:500 for anti-human Flt-1 antibody, 1:500 for anti-human KDR/Flk-1 antibody, 1:1000 for anti-human Bcl-xL antibody, 1:500 for anti-human Bcl-2 antibody, 1:400 for anti-mouse Bax antibody, 1:500 for anti-human cyclin D1 antibody, 1:300 for anti-human p27 antibody, and 1:500 for anti-human p21 antibody from Santa Cruz Biotechnology (Santa Cruz, CA), and 1:1000 for anti-human β-actin from Sigma (St Louis, MO), and 1:2000 for all secondary antibodies. The protein band intensities were analyzed densitometrically with the values for each protein band expressed relative to the density of the actin band.