研究成果の刊行に関する一覧表

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なし

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IV. 研究成果の刊行物・別刷

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Phase I/II Study of S-1 plus Cisplatin Combination Chemotherapy in Patients with Advanced/Recurrent Head and Neck Cancer

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Objective: The objectives of this study were to determine the maximum tolerated dose (MTD) and recommended dose (RD) of S-1 plus cisplatin (CDDP) and to evaluate safety and efficacy using the defined RD in advanced/recurrent head and neck cancer (HNC).
Methods: S-1 was administered orally at 40 mg/m^2 twice daily for 14 consecutive days, and CDDP was infused on day 8 at a dose of 60 and 70 mg/m^2. Each course was repeated every 4 weeks.
Results: A total of 38 patients were registered, 10 patients for the Phase I study and an additional 28 patients for the Phase II study. Although no dose-limiting toxicity (DLT) was observed in the CDDP 60 mg/m^2 (Level 1) group, two of six patients in the CDDP 70 mg/m^2 (Level 2) group exhibited DLT (fatigue/diarrhea). The MTD was not achieved in the Phase I study. Level 2 was therefore determined as the RD. In the Phase II study, 34 patients, including 6 patients from the Phase I study, were evaluated. At the termination of treatment, the confirmed response rate was 44.1% (15/34, 95% CI: 27.4–60.8). The best response rate without an adequate duration time was 67.6% (95% CI: 51.9–83.4). The median survival period was 16.7 months, and the 1-year survival rate was 60.1%. The main toxicities of Grade 3 or above were anorexia (26.5%), nausea (14.7%), neutropenia/thrombocytopenia (11.8%) and anemia/fatigue (8.8%).
Conclusions: This is considered to be an effective regimen with acceptable toxicities for HNC.

Key words: head and neck cancer – S-1 – CDDP – chemotherapy

INTRODUCTION

As clinical characteristics of head and neck cancer (HNC), ~90% of the cases are squamous cell carcinoma, and two-thirds of the patients suffer from locoregional advanced (Stages III and IV) disease. Although the prognosis for early-stage (Stages I and II) HNC is satisfactory with 5-year survival rates of 70–90% after standard therapy such as surgery, radiotherapy or both (1), the 5-year survival rate falls to <50% in the locoregional advanced stage, even if radical treatment such as surgery, radiotherapy or chemotherapy [at the induction/concurrent chemoradiotherapy (CCRT)] is performed.

For patients suffering from incurable cancer or recurrent disease, either locoregionally or in the form of distant

metastasis, the prognosis is particularly poor, with a median survival period of only 6 months with conventional palliative chemotherapy (1). Some combination therapies, including cisplatin (CDDP), were devised after Wittes et al. (2) reported the efficacy of CDDP for HNC in 1977, and the efficacy became clear (3,4). Since Kish et al. (5) reported the efficacy of CDDP plus 5-fluorouracil (5-FU) combination therapy (CDDP/5-FU) in 1982, moreover, CDDP/5-FU has been considered the most common combination chemotherapy, and it has been widely employed as the first-line chemotherapy for advanced/recurrent HNC. The response rate for CDDP/5-FU has been reported to be 50–90% (6–8) when used as the first-line induction chemotherapy and to be 32–48% (3,9) when used as second-line or later recurrent chemotherapy. CDDP/5-FU requires long-term hospitalization, however, because it involves continuous infusion of 5-FU and requires adequate support for mucosal and renal toxicity.

S-1 (Taiho Pharmaceutical Co., Ltd, Tokyo, Japan) is a novel oral anticancer agent consisting of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) at a molar ratio of 1:0.4:1, based on biochemical modulation of 5-FU (10). S-1 showed high response rates of 28.8–46.2% with acceptable toxicity in Phase II studies for advanced/recurrent HNC conducted in Japan (11,12). It was approved for HNC in Japan under the approval regulation system in 2001. If the efficacy and toxicities of S-1 plus CDDP combination therapy (S-1/CDDP) were similar to those of CDDP/5-FU in this study, it is thought that it would become one of the potential choices as chemotherapy for advanced/recurrent HNC.

PATIENTS AND METHODS

Patient Selection

The following eligibility criteria were used: histologically or cytological confirmed HNC (excluding thyroid cancer), unresectable locally advanced (Stage III/IV disease) and recurrent or distant metastasis, at least measurable disease after prior treatment. If the patients had received prior treatment, radiotherapy more than 28 days, surgery and chemotherapy or adjuvant chemotherapy more than 14 days was required before registration. Other eligibility criteria included the following: age 20–80 years, Eastern Cooperative Oncology Group (ECOG) performance status 0–1, life expectancy >3 months, adequate bone marrow, hepatic and renal functions (reflected by an absolute hemoglobin level of >9.0 g/dl, leukocyte count > lower limit of normal, platelet count $>100 \times 10^{-9}$ cells/l, normal bilirubin level of <1.5 mg/dl, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels <2.5 times the upper limit of normal, serum creatinine level < upper limit of normal and creatinine clearance >70 ml/min). Within 14 days before registration, all patients underwent a complete physical examination that included their medical history, blood count, serum biochemistry tests (hepatic and renal function tests and electrolytes), urinalysis and echocardiography; a chest radiograph (X-ray) and computed tomography (CT) or magnetic resonance imaging scans of all disease sites were obtained during the 28 days before registration. Patients who had undergone induction chemotherapy with CDDP/5-FU or platinum-based chemotherapy were excluded from the Phase II study unless it used a lower dose as a sensitizer of CCRT. The exclusion criteria were as follows (summary): severe drug hypersensitivity, pulmonary fibrosis or interstitial pneumonia, severe heart disease, difficult-to-control diabetes, active infection or acute inflammation, active concomitant malignancy, and other serious medical conditions. Patients were required to give written informed consent before admission to the study. The study protocol was approved by the instruction review board of each participating hospital, and the study was conducted in accordance with the Japanese Good Clinical Practice guideline.

Treatment and Dose-escalation Schedule

Phase I Study

S-1 was administered orally at the dose of 40 mg/m^2 twice a day, after the morning and evening meals between days 1 and 14. Three initial doses were established according to the body surface area (BSA): <1.25 m^2, 80 mg/day; 1.25–1.5 m^2, 100 mg/day; >1.5 m^2, 120 mg/day. CDDP was administered intravenously over 2 h on day 8. The treatment was repeated every 4 weeks. The starting dose of CDDP was 60 mg/m^2 as Level 1 with a planned increase to 70 mg/m^2 as Level 2. We did not establish a Level 3, because the approved dose of CDDP in Japan is 70 mg/m^2. For the first step, three patients were treated at the Level 1 dose. They would then go to Level 2 if no patient showed dose-limiting toxicity (DLT). If one or two of the three patients in the first step showed any DLT, three additional patients were to be enrolled at the same dose level. A dose level would be determined as maximum tolerated dose (MTD) if more than three of six patients showed DLT. The recommended dose (RD) was to be one level below the MTD level.

Phase II Study

A treatment regimen with the RD determined in the Phase I study was repeated every 4 weeks at least two cycles unless progression or unacceptable toxicity occurred. The next course was started for patients whose organ biological parameters had been maintained at the level of the eligibility criteria (leukocyte count >3000 mm^{-3}, platelet count $>75 \times 10^{-9}$ cells/l and non-hematologic toxicity >Grade 2).

If these criteria were satisfied 3 weeks after day 1 of each cycle of chemotherapy, the next cycle could be administered. The doses of S-1 were adjusted according to the degree of hematologic and non-hematologic toxicities. The dose was reduced by one level, 20 mg/day, in patients whose BSA was

$>1.25\ m^2$, with evidence of Grade 4 hematologic toxicity or Grade 3 or greater non-hematologic toxicity during any phase of the administration cycle. If a patient with a BSA of $<1.25\ m^2$ experienced the above toxicities, no further treatment with S-1 was conducted. If treatment was stopped for ≥ 4 weeks, the patient was withdrawn from the study.

Definitions of DLT and MTD

Toxicity was evaluated according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC), version 2.0. DLT was defined as Grade 4 leukocytopenia or thrombocytopenia, Grade 4 neutropenia lasting for 4 days, occurrence of neutropenic fever ($\geq 38°C$) with Grade 3 leukocytopenia or neutropenia, any Grade 3 non-hematologic toxicity except nausea, vomiting or anorexia following related events occurring in the first course. Patients were also categorized in the DLT group when the second course of treatment was not resumed within 21 days after the first course. The MTD was defined as the dose at which 50% (3/6) or more patients experienced DLT during the first course.

Evaluation and Follow-up

Examinations of blood chemistry and symptoms of toxicity were repeated weekly. The clinical response was measured for each course based on the CT scans or X-ray findings that initially had been used to define the tumor extent. Toxicities were graded according to the NCI-CTC, version 2.0. Tumor responses were evaluated according to the criteria of the World Health Organization (1979), which was an evaluation standard in Japan at the time of the start of this study. Complete response (CR) was defined as the disappearance of all measurable and assessable diseases for at least 4 weeks. Partial response (PR) was defined as a $\geq 50\%$ reduction in the sum of the products of the largest diameters of the measurable disease for at least 4 weeks. Stable disease (SD) was defined as failure to observe a PR or CR or progression of the disease for at least 4 weeks. Progressive disease (PD) was defined as a $\geq 25\%$ increase in the sum of the products of the largest diameters of the measurable disease or the appearance of new lesions. We conducted assessment meetings for mutual assessment of patient's eligibility and their response to treatment.

Statistical Analysis

The primary objective of the Phase I study was to determine the MTD and RD, and the secondary objective was evaluation of the safety. In the Phase II study, the primary objective was to evaluate efficacy using the defined RD, and the secondary objective was evaluation of the safety and survival.

The number of the Phase II study patients to be enrolled in this study was calculated as ≥ 26, which was required to offset the null hypothesis that the lower bound of 95% CI of the expected response rate (65%) would be $<35\%$ under conditions of an α error of 0.05 and a β error of 0.2. The overall survival of eligible patients was defined from the start of treatment to death or the last follow-up visit and was estimated by the Kaplan–Meier method.

RESULTS

Between July 2002 and June 2004, 10 patients were entered in the Phase I study and 28 were entered in the Phase II study to confirm the efficacy and toxicities at the RD.

All patients were eligible for the toxicity evaluation for the total course and for objective response evaluations (Table 1). In the Phase I study, because one patient might have contravened the exclusion criteria, active infection or acute inflammation after registration, four patients were enrolled in the Level 1 group. In the Phase II study at the RD including six patients of the Phase I study, 18 patients with unresectable advanced HNC were enrolled, which included 1 patient with distant metastasis (lung and liver). Twenty patients with recurrent HNC included five patients with distant metastasis (lung, skin and bone) who had received prior therapy (surgery, radiation, chemotherapy or more than one) and 10 patients had previously received CCRT with the docetaxel or platinum anticancer agents (carboplatin and CDDP) and other anticancer agents (5-FU, tegaful/uracil and methotrexate). A total of 75 courses were administered: a total of 6 courses at Level 1 in the Phase I study (median: 2 courses, range: 1–2) and a total of 69 courses at the RD (median: 2 courses, range: 1–3).

Determination of MTD and DLT

The toxicities (drug-related adverse events) observed during the first course are shown in Table 2. DLT was not observed at Level 1. At dose level 2, two of six patients showed DLT, one of them Grade 3 diarrhea and the other Grade 3 fatigue. The MTD was not achieved in the Phase I study. Dose level 2 was therefore determined to be RD in the subsequent Phase II study according to the provisions stated in the protocol.

Safety

The toxicities observed among 34 patients, including 6 patients in Level 2 in the Phase I study, are shown in Table 2. The most common toxicities and incidences were hematologic toxicities (64.7–94.1%), gastrointestinal (GI) dysfunction (79.4–82.4%) and fatigue (58.8%). The toxicities of Grade 3 or above observed were anorexia (26.5%), nausea (14.7%) and fatigue (8.8%). The hematologic toxicities of Grade 3 or above observed were 11.8% in this study, and granulocyte colony-stimulating factor was administered to one patient. The number of confirmed treatment-related

Table 1. Patient characteristics

	Phase I study		Phase II study
CDDP (mg/m^2)	60	70	70
No. of patients	4	6	28
Gender			
Male	3	4	25
Female	1	2	3
Age (years)			
Median	57.0	65.5	61.5
Range	49–68	49–73	33–73
Performance status			
0	4	5	20
1	0	1	8
Pathology type			
SCC	4	6	28
Primary disease site			
Oral cavity	1	1	9
Nasopharynx	1	1	3
Oropharynx	1	2	3
Hypopharynx	0	1	6
Larynx	0	0	4
Nasal cavity/paranasal sinus	1	1	2
External acoustic meatus	0	0	1
Classification			
Advance disease	0	3	15
Stage IIb	0	1	0
Stage III	0	1	5
Stage IV	0	1	10
TNM:M1 (lung + liver)	0	0	1
Recurrent disease	4	3	13
Locoregional	3	3	8
Regional lymph node	1	2	4
Distance metastasis (bone)	1	1 (1)	3
Prior treatment			
None	0	3	15
Surgery	1	2	10
Radiation (CCRT)	4 (4)	3 (0)	10 (6)
Chemotherapy (adjuvant)	4 (1)	3 (2)	6 (2)
Assessable (target) lesion			
Locoregional	3	6	22
Regional lymph node	1	4	12
Distance metastasis	1	0	4
Lung	1	0	3
Liver	0	0	1
Skin	0	0	1

CDDP, cisplatin; SCC, squamous cell carcinoma; CCRT, concurrent chemoradiotherapy.

deaths in this study was one, a patient who died of pneumonia accompanied by sepsis for Grade 3 leukocytopenia.

Response and Survival

A total of 34 patients were evaluated to determine the response rate at the RD (Tables 3 and 4).

The confirmed response rate (C-RR) with >4-week duration was 44.1% (15/34, 95% CI: 27.4–60.8). There were 2 CRs (5.9%), 13 PRs included 3 distant metastasis which was 2 lungs and skin (PR: 38.2%), 15 cases of SD (44.1%) and 3 cases of PD (8.8%). In the subgroup analysis, the C-RRs per classification were advanced HNC 44.4% (8/18, 95% CI: 24.6–66.3) and recurrent HNC 43.8% (7/16, 95% CI: 19.4–68.1).

The median time to progression in the Phase II study (28 patients) was 100 days (range: 70–140). The median time to PR (50% tumor reduction) and the median overall duration of response in 11 responding patients were 25 days (range: 17–56) and 61 days (range: 38–116), respectively.

We additionally considered the best responses, including patients with a duration time of <4 weeks. The best overall response was 67.6% (95% CI: 51.9–83.4). Among the best responses, there were seven cases of CR (20.6%), 16 of PR (47.1%), seven of SD (20.6%) and three of PD (8.8%). In the subgroup analysis, the best overall responses per classification were advanced HNC 72.2% (13/18, 95% CI: 51.5–92.9) and recurrent HNC 62.5% (10/16, 95% CI: 38.8–86.2).

The median survival time (MST) of 34 patients was 16.7 months (95% CI: 11.4–no data), whereas the 1-year survival rates were 60.1% (Fig. 1). In the subgroup analysis, the 1-year survival rate for advanced HNC was 83.0%, and MST for recurrent HNC was 9.8 months (95% CI: 7.5–13.3) and the 1-year survival rate was 34.4% (Fig. 2). The median follow-up time for survival analysis was 13.1 months (range: 1.84–28.4). The detail of the treatment for 18 patients with advanced HNC after this regimen end was as follows: 8 patients received CCRT, 6 received radiotherapy, 2 received surgery and 2 were untreated. In addition, for 16 patients with recurrent cancer, 2 patients received CCRT, 2 received radiotherapy, 1 received surgery, 5 received adjuvant chemotherapy and 6 were untreated.

Discussion

S-1 is a novel oral anticancer agent consisting of FT, CDHP and Oxo at a molar ratio of 1:0.4:1. FT is a prodrug of 5-FU which is gradually converted to 5-FU and rapidly catabolized by dihydropyrimidine dehydrogenase (DPD) in the liver. CDHP inhibits the catabolism of 5-FU released from FT by DPD. CDHP helps maintain efficient blood and tumor concentrations of 5-FU at much the same levels as continuous infusion of 5-FU. Oxo was selected as a modulator that inhibits phosphorylation of 5-FU in the digestive mucosal cells and that inhibits phosphoribosylaton of 5-FU in the GI mucosa. It achieved high efficacy without increasing GI

Table 2. Toxicity incidence

	Phase I study (first course)										Phase II study/Level 2 (all courses)				
Level	Level 1					Level 2									
CDDP (mg/m^2)	60					70					70				
No. of patients (*n*)	4					6					34				
Toxicities/grade	Grade				>Grade 3	Grade				>Grade 3	Grade				>Grade 3
	1	2	3	4		1	2	3	4		1	2	3	4	
Hematologic toxicity															
Anemia	2	1	1	0	25.0	4	0	1	0	16.7	15	9	2	1	8.8
Leukocytopenia	0	2	0	0	0.0	2	2	0	0	0.0	9	9	3	1	11.8
Neutropenia	0	1	1	0	25.0	2	2	0	0	0.0	8	14	2	2	11.8
Thrombocytopenia	2	0	0	0	0.0	6	0	0	0	0.0	27	1	3	1	11.8
Non-hematologic toxicity															
Anorexia	0	2	1	0	25.0	1	2	3	0	50.0	10	8	8	1	26.5
Nausea	0	1	0	0	0.0	3	1	1	0	16.7	14	9	5	0	14.7
Vomiting	0	0	0	0	0.0	2	1	0	0	0.0	8	3	0	0	0.0
Mucositis	0	0	0	0	0.0	1	2	0	0	0.0	7	5	0	0	0.0
Diarrhea	1	0	0	0	0.0	1	0	1	0	16.7	6	3	1	0	2.9
Fatigue	1	0	0	0	0.0	1	2	1	0	16.7	11	6	2	1	8.8
Alopecia	0	0	0	0	0.0	3	0	0	0	0.0	5	0	0	0	0.0
Rash	1	0	0	0	0.0	1	0	0	0	0.0	5	3	0	0	0.0
Hyperpigmentation	1	0	0	0	0.0	1	1	0	0	0.0	8	2	0	0	0.0

Table 3. Objective response rate at the RD

	No. of patients	CR	PR	SD	PD	NE	Response rate (%)	95% CI (%)
Phase I study								
Level 2	6	1	3	1	1	0	66.7	—
Phase II study								
RD	28	1	10	14	2	1	39.3	21.5–59.4
Total	34	2	13	15	3	1	44.1	27.4–60.8

RD, recommended dose; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; CI, confidence interval.

toxicity, based on biochemical modulation of 5-FU (10). S-1 was consequently approved in Japan for gastric cancer under an accelerated approval regulation system in 1999 (13,14) and subsequently for HNC in 2001 (11,12), colorectal cancer in 2003 (15,16), non-small lung cancer (NSCLC) in 2004 (17,18), breast cancer in 2006 (19), and pancreatic cancer (20,21) and biliary tract cancer in 2007 (22), and clinical trials of its use for renal cell cancer, prostate cancer, liver cancer and cervical cancer are currently under way. In other carcinomas, S-1/CDDP was carried out for advanced gastric cancer (AGC) and NSCLC with response rates of 76% (23) and 47% (24), and acceptable toxicities. In addition, a randomized Phase III study for AGC patients as the first-line chemotherapy, the SPIRITS study, was reported and proved the superiority of S-1/CDDP to S-1 monotherapy (25). The response rate for combination therapy versus monotherapy was 54.0% versus 31% ($P = 0.0018$), and the MST was 13.0 versus 11.0 months (median follow-up time 34.6 months; hazard ratio 0.774; $P = 0.0366$).

This study and other studies on combination therapy for HNC with S-1 plus CDDP analogues, S-1 plus carboplatin combination therapy (S-1/carboplatin) (26) and S-1 plus nedaplatin combination therapy (S-1/nedaplatin) (27) have been conducted since 2002, and the results of these studies have been reported. S-1/CDDP (75 mg/m^2) was also performed in Korea as the first-line remission induction therapy for advanced clinical Stage III/ IV cancer, and a high response rate of 89.7% was reported (28).

In this study of S-1/CDDP administration for advanced/recurrent HNC performed this time, the toxicities observed were mild and acceptable in light of the safety of this combination therapy. As concerns efficacy in this study, the C-RR was 44.1% (95% CI: 27.4–60.8), which was lower than the expected response rate of 65% to <35% under these

Table 4. Objective response rate at the RD (subgroup analysis)

Classification	No. of patients	Response	CR	PR	SD	PD	NE	Response rate (%)	95% CI (%)
Advanced	18	Best over all response	5	8	3	1	1	72.2	51.5–92.9
		Confirmed response	1	7	8	1	1	44.4	24.6–66.3
Recurrent	16	Best over all response	2	8	4	2	0	62.5	38.8–86.2
		Confirmed response	1	6	7	2	0	43.8	19.4–68.1
Total	34	Best over all response	7	16	7	3	1	67.6	51.9–83.4
		Confirmed response	2	13	15	3	1	44.1	27.4–60.8

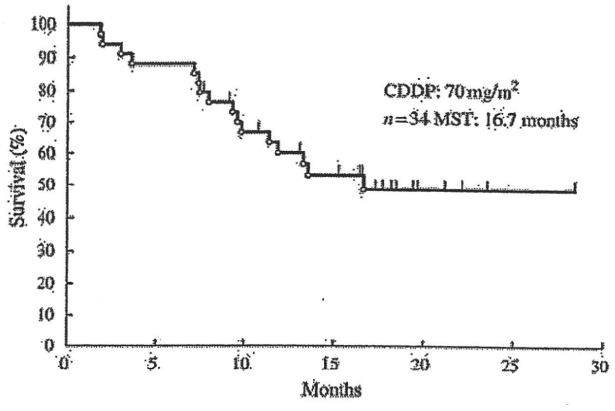

Figure 1. Overall survival.

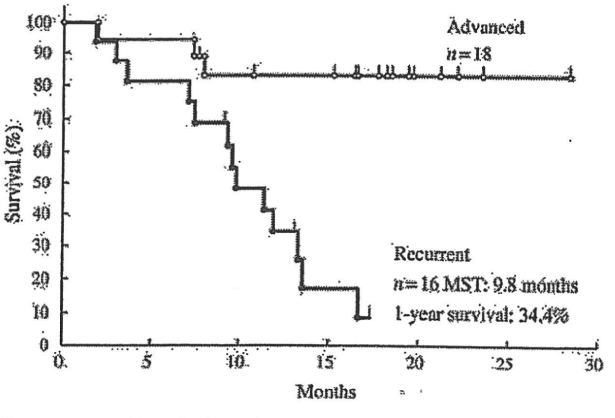

Figure 2. Overall survival of subgroups (advanced and recurrent).

conditions. Evaluation of the C-RR according to advanced HNC accounted for 44.4% and recurrent HNC accounted for 43.8%. The response rate after second-line therapy for recurrent cancer was comparable to the following reported results: 32–48% for 5-FU/CDDP (3,9), 40% for docetaxel/CDDP combination therapy (docetaxel/CDDP) (29) and 41.1% for paclitaxel/CDDP combination therapy (paclitaxel/CDDP) (30) for studies performed overseas, and 40.9% for S-1/carboplatin (26) and 33.3% for S-1/nedaplatin (27) for studies performed in Japan.

The response rate for S-1/CDDP for advanced cancer was lower than the response rates (50–90%) for 5-FU/CDDP (6–8). Suggested reasons for the lower response rate for advanced cancer included the short duration of the average of two administration courses (28) and the involvement of the treatment system for locoregional advanced cancer. The treatment system for locoregional advanced cancer was specified as follows. If chemotherapy is performed as prior treatment, perform two target courses of the therapy and make a decision on the form of treatment to follow according to the therapeutic efficacy at the end of the second course. If the effectiveness evaluation is SD at the end of one course of the preceding chemotherapy, perform radiotherapy or surgery with the addition of postoperative radiotherapy. If PR (50% tumor reduction) is achieved at the end of one course of the preceding chemotherapy, add a second course. If PR continues at the end of second course, perform radiotherapy or surgery with the addition of post-operative radiotherapy; and if CR is achieved, add another course of chemotherapy with radiation irradiation to complete the treatment. As an exception in this study, when a tumor achieved reduction of >50%, we considered the benefit to the patient and allowed the next treatment to be conducted based on the judgment of the doctor. In this study, three of the eight patients with advanced HNC who were evaluated as SD in C-RR showed tumor reduction of >50%, as in the case of five patients administered a second therapy (radiotherapy, chemoradiotherapy) without waiting for the 28-day duration of effect with patient benefit taken into consideration. Considering the action of these results, the best response rate for advanced cancer was 77.2% (95% CI: 51.5–92.9) in this study.

The MST in this study was 16.7 months, and the 1-year survival rate was 60.1%. MST in recurrent cancer was 9.8 months with a 1-year survival rate of 34.4%. These results were comparable to those for cetuximab/CDDP (33), gefitinib/methotrexate (34), docetaxel/CDDP (29), paclitaxel/CDDP (30) and S-1/carboplatin (26). In advanced cancer, the 1-year survival rate was 83.0%, although the observation period was short, and this result was closely similar to that for 5-FU/CDDP (6–8).

As concerns safety, the most common toxicities in this study were hematologic toxicity and GI dysfunction and

fatigue, and the hematologic toxicity was mild when compared with the results for conventional 5-FU/CDDP (3,6–9), with an incidence of 11.8%.

The results of this study indicated that S-1/CDDP can be considered to be effective with acceptable toxicities for advanced/recurrent HNC. With respect to recurrent HNC, it was determined that a Phase III comparative study with a CDDP base was necessary, among other things. As concerns locoregional advanced HNC, it is desirable to carry out CCRT. S-1 exhibits radiosensitization action and it is often used in combination with radiation therapy. Tahara et al. (31,32) conducted a Phase I study of CCRT with S-1/CDDP for unresectable advanced HNC and suggested its utility. We plan to conduct a Phase II multicentred trial as a JCOG study.

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Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Weekly paclitaxel in patients with recurrent or metastatic head and neck cancer

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Abstract
Purpose To evaluate the efficacy and safety of weekly paclitaxel in patients with recurrent or metastatic head and neck cancer (HNC) by combined analysis of early and late phase II trials.
Methods Eligibility criteria included histologically proven HNC with recurrent or metastatic disease, measurable disease, PS 0-2, and one or no prior chemotherapy regimens. Treatment consisted of a 1-h infusion of paclitaxel at a dose of 100 mg/m^2 weekly for 6 weeks of a 7-week cycle. A total of 74 patients were enrolled: 37 between February and November 2004 in an early phase II trial and 37 between October 2005 and July 2006 in a late phase II trial.
Results The median number of treatment cycles was two, and median dose intensity was 84.2 mg/m^2/week. The most common grade 3–4 adverse events were leukopenia (37.5%), neutropenia (30.6%), anemia (12.5%), constipation (8.3%), peripheral neuropathy (5.6%), anorexia (5.6%), and pneumonitis (5.6%). Overall response rate was 29.0% according to RECIST. The median duration of response, median time to progression, and median survival time were 7.4, 3.4, and 14.3 months, respectively.
Conclusions This study demonstrates that weekly paclitaxel has promising activity with acceptable toxicity in the treatment of recurrent or metastatic HNC.

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Keywords Paclitaxel · Head and neck cancer · Phase II study · Weekly infusion

Introduction

Head and necks cancers (HNCs) are the sixth most common cancers worldwide, and approximately 500,000 new cases are projected annually [22]. An estimated 60% of these patients present with locally advanced disease (stage III/IV) [32]. Although the treatment of these locally advanced HNC has progressed, half will recur. While some of these are suitable for salvage treatment, including surgery or chemoradiotherapy, most are scheduled to receive palliative chemotherapy only.

Platinum-based combination chemotherapy is widely used as first-line treatment for recurrent/metastatic HNC. However, while several randomized trials have suggested that combination chemotherapy yields superior response rates, it is also associated with increased toxicity and no significant survival advantage over single agent chemotherapy [1, 4, 5, 15, 31, 35]. A recent randomized trial of platinum-based chemotherapy with or without cetuximab demonstrated significant survival benefit in the arm receiving cetuximab [30]. However, cetuximab was not given to patients in the control arm at the time of progression and it therefore remains unanswered whether the addition of cetuximab to first-line chemotherapy provides a survival benefit over sequential use of platinum-based chemotherapy followed by cetuximab at the time of progression. In other words, standard therapy in first-line treatment for recurrent/metastatic HNC has not yet been established. Furthermore, treatment options for patients who are refractory to platinum-based chemotherapy are limited. Optimal treatment options for these patients are therefore desirable.

Paclitaxel is a novel diterpenoid isolated from the bark of the Pacific yew, *Taxus brevifolia* [34]. Paclitaxel has high-affinity binding to microtubules, promotes microtubule assembly, and stabilizes tubulin polymers against depolymerization affecting cells in the G2/M-phase [24, 26].

Previous studies of high-dose tri-weekly paclitaxel (200–250 mg/m^2) in patients with advanced or recurrent/metastatic HNC demonstrated treatment activity, with an overall response of 35–40%, but that this regimen was associated with severe neuropathy and myelosuppression [6, 27]. Since the survival of patients with recurrent or metastatic HNC is limited, additional consideration should be given to their quality of life.

Previous studies of weekly paclitaxel at a reduced single dose for other cancers demonstrated comparable efficacy to a high-dose tri-weekly regimen with milder toxicities, including neuropathy and myelosuppression [28].

At the time the present trials were planned, only one prospective phase II study of weekly paclitaxel in the treatment of recurrent or metastatic HNC had appeared. Results showed acceptable toxicities but the poor response rate of 9.3% (4/43) [3]. Thus, no data were available to support the practical use of weekly paclitaxel in the treatment of recurrent or metastatic HNC, albeit that weekly paclitaxel has been widely used in the treatment of HNC patients who are refractory to a platinum-based chemotherapy.

Here, therefore, we conducted two multicenter, phase II trials, an early and late phase II trial of weekly paclitaxel in patients with recurrent or metastatic HNC, to evaluate efficacy and safety in the two trials and to confirm data on safety and efficacy between them.

Patients and methods

The subjects of the present study were patients enrolled in two multicenter trials, an early and a late phase II trial of weekly paclitaxel in the treatment of recurrent or metastatic HNC. To allow the safety and efficacy of these trials to be compared, they were conducted under the same design. Each trial was conducted at 19 institutions in Japan.

Eligibility criteria included histologically or cytologically proven HNC with recurrent or metastatic disease; age 20 years or older but less than 75; a measurable lesion; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2; adequate organ function, as defined by an absolute neutrophil count (ANC) >2,000/μL, platelet count >100,000/μL, hemoglobin >9.0 g/dL, AST <100 IU/L, ALT <100 IU/L, total bilirubin <1.5 mg/dL, and serum creatinine <1.5 mg/dL; and life expectancy >2 months from the beginning of treatment. Patients were excluded if they had received two or more prior regimens of chemotherapy for recurrent/metastatic HNC. The study protocol was reviewed and approved by the ethics committee of each of the participating institutions before patient enrollment began. Informed consent was obtained from all patients.

Treatment

On the basis of the results of a phase I trial of weekly paclitaxel in solid tumors [20], patients in both the early and late phase trials received a 1-h iv infusion of paclitaxel at a dose of 100 mg/m^2 weekly over a 7-week cycle on days 1, 8, 15, 22, 29, and 36, followed by 2 weeks of rest until unacceptable toxicity, patient refusal, or disease progression were observed. Patients received premedication with 8 mg dexamethasone (iv), 50 mg ranitidine (iv),

and 50 mg diphenhydramine hydrochloride (po) 30–60 min prior to paclitaxel infusion.

Dose modification of paclitaxel by 20 mg/m^2 was allowed if a patient experienced any of the following adverse events: (1) febrile neutropenia, (2) grade 3 or 4 thrombocytopenia, (3) grade 3 or 4 non-hematological toxicity, (4) grade 2 or higher peripheral neuropathy or myalgia/arthralgia, or (5) any toxicity that caused a dose to be skipped or required a dose reduction at the discretion of the physician. Dose reduction to less than 60 mg/m^2 was not allowed.

Study endpoints

The primary endpoints in each trial were safety and response rate as assessed by WHO criteria, which could be compared to historical data. Secondary endpoints were duration of response, response rate based on the response evaluation criteria in solid tumors (RECIST), median time to progression (TTP), and median survival time (MST). The response rates and adverse events were evaluated by an independent safety and efficacy assessment committee. Responses were assessed by CT and/or MRI scans every 4 weeks. Adverse events were evaluated every week according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 2.0. A subject's TTP was defined as the time from the date of the enrollment in the present study to the first documentation of disease progression, subsequent therapy, or death. The duration of response was defined as the time from the date of the first confirmation of response to the first documentation of disease progression.

Statistical design

To confirm safety and efficacy, applications for approval of anti-neoplastic drugs in Japan typically require two studies conducted under the identical design, an early and a late phase II trial. If the early trial does not demonstrate promising activity, the late trial is withheld. In each of the present studies, the expected response rate was considered to be 25% and the threshold response rate was set at 10%. Thirty-six patients were needed to evaluate efficacy in each study in order to reject the hypothesis that the true efficacy rate was below the threshold response rate, giving $\alpha = 0.025$ (one-sided) and $\beta = 0.3$. A survival curve was estimated using the Kaplan–Meier method [16]. In the present trials, safety and efficacy analyses were conducted on an intention-to-treat (ITT) population, defined as all patients enrolled in the study who received at least one dose of paclitaxel. All statistical analyses were carried out using SAS Version 8.2.

Results

Patient characteristics

A total of 74 patients were enrolled, 37 between February and November 2004 in the early phase II trial and 37 between October 2005 and July 2006 in the late phase II trial. The two trials had one patient each who did not receive any administration of paclitaxel due to PS 3 or ANC <2,000/μL. Patient characteristics are shown in Table 1. Of note, a total of 25 (34.7%) patients had advanced cancer, 47 (65.3%) had recurrent cancer, and 62 (86.1%) had a prior history of chemotherapy, including platinum-based chemotherapy (76.4%). Of these, 23 (31%) had received prior platinum-based chemotherapy for recurrent/metastatic disease. No relevant differences in patient characteristics were observed between individuals in the early and late phase trial groups.

Treatment administration

For both the early and late phase trials, the combined median number of treatment cycles was 2.0 (range 1–10) and the median number of doses was 12 (range 1–50). The combined median interval between cycles was 14.0 days (range 13–28 days), and the median dose intensity was 84.2 mg/m^2/week (range 43.0–107.7 mg/m^2/week).

Safety

The safety evaluation was conducted in 72 patients who received at least one dose of paclitaxel. Adverse events are shown in Table 2. The most common grade 3–4 non-hematological adverse events were constipation (8.3%), peripheral neuropathy (5.6%), anorexia (5.6%), and pneumonitis (5.6%), while grade 3–4 hematological adverse events were leukopenia (37.5%), neutropenia (30.6%), and anemia (12.5%). No deaths related to paclitaxel treatment were seen during the study period. The incidence of greater than grade 2 peripheral neuropathy was 25.0% (18/72).

The percentage of patients requiring dose reductions was 34.7% (25/72). Although 16.7% (12/72) of patients required cessation of therapy, only 5.6% (4/72) was unable to complete the protocol of at least one cycle of paclitaxel. The most common reason for cessation was peripheral neuropathy, seen in 6.9% (5/72) of patients. The median time to onset of peripheral neuropathy was 34 days (range 1–141), and the median dose of onset was 500 mg/m^2 (range 100–1600 mg/m^2). In those patients who experienced peripheral neuropathy, 14.5% (8/55) recovered, 7.3% (4/55) remitted, and 78.2% (43/55) failed to recover by the end of the protocol.

Table 1 Patient characteristics

Characteristics	Number of subjects (%)		
	Total, $n = 72$	Early phase II study, $n = 36$	Late phase II study, $n = 36$
Sex			
Male	56 (77.8)	30 (83.3)	26 (72.2)
Female	16 (22.2)	6 (16.7)	10 (27.8)
Age			
Median age (range)	61 (41–74)	60.5 (44–74)	62.5 (41–74)
P.S. (ECOG)			
0	48 (66.7)	22 (61.1)	26 (72.2)
1	22 (30.6)	13 (36.1)	9 (25.0)
2	2 (2.8)	1 (2.8)	1 (2.8)
Disease status			
Advanced	25 (34.7)	10 (27.8)	15 (41.7)
Recurrent	47 (65.3)	26 (72.2)	21 (58.3)
Histopathological diagnosis			
Squamous cell carcinoma	61 (84.7)	32 (88.9)	29 (80.6)
Adenoid cystic carcinoma	4 (5.6)	1 (2.8)	3 (8.3)
Others	7 (9.7)	3 (8.3)	4 (11.1)
Primary lesion			
Oral cavity	8 (11.1)	8 (22.2)	0
Paranasal cavity	8 (11.1)	3 (8.3)	5 (13.9)
Nasopharynx	8 (11.1)	4 (11.1)	4 (11.1)
Oropharynx	12 (16.7)	6 (16.7)	6 (16.7)
Hypopharynx	18 (25.0)	7 (19.4)	11 (30.6)
Larynx	6 (8.3)	3 (8.3)	3 (8.3)
Salivary gland	7 (9.7)	1 (2.8)	6 (16.7)
Others	5 (6.9)	4 (11.1)	1 (2.8)
Prior treatment			
Chemotherapy*	62 (86.1)	32 (88.9)	30 (83.3)
Cisplatin-based chemotherapy	55 (76.4)	29 (80.6)	26 (72.2)
Others	7 (9.7)	3 (8.3)	4 (11.1)
Surgery	36 (50.0)	20 (55.6)	16 (44.4)
Radiotherapy	60 (83.3)	30 (83.3)	30 (83.3)

PS performance status, *ECOG* Eastern Cooperative Oncology Group

* Including adjuvant chemotherapy, neoadjuvant chemotherapy, and chemoradiotherapy

Efficacy

Thirty-six patients in each study were assessed for efficacy (Table 3). Overall response rates (RRs) in the early and late trial were 33.3% (95% CI: 18.6, 51.0%) and 36.1% (95% CI: 20.8, 53.8%), respectively. In combined analysis of two trials, RR according to WHO and RECIST criteria were 34.7% (95% CI: 23.9, 46.9%) and 29.0% (95% CI: 18.7, 41.2%), respectively. RR according to the WHO criteria in the 55 patients who received prior platinum-based chemotherapy was 32.7% and 30.4% in the 23 patients who received prior platinum-based chemotherapy for recurrent/metastatic disease (Table 4). RR in the 60 patients who received prior radiotherapy, including adjuvant therapy, neoadjuvant therapy, and chemoradiotherapy, was 30.0 and 58.3% in the 12 patients who did not receive prior radiotherapy.

The median duration of response was 8.5 months (95% CI: 5.4, 11.5 months) in the early trial, 6.9 months (95% CI: 3.2, 7.9 months) in the late trial, and 7.4 months (95% CI: 5.4, 9.4 months) in total.

The median follow-up period in all patients was 13.8 months (range: 1.6-33.8 months). Median TTP and MST were 3.4 months (95% CI: 3.0, 4.6 months; Fig. 1) and 14.3 months (95% CI: 11.0, 19.4 months; Fig. 2), respectively. In the 64 patients excluding those with nasopharyngeal cancer, median TTP and MST were 3.2 months (95% CI: 2.9, 4.3 months) and 13.0 months

Table 2 Adverse events

	Total ($n = 72$)				Early phase II study ($n = 36$)				Late phase II study ($n = 36$)			
	≥Grade 1		≥Grade 3		≥Grade 1		≥Grade 3		≥Grade 1		≥Grade 3	
	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
Nausea	22	30.6	2	2.8	9	25.0	1	2.8	13	36.1	1	2.8
Anorexia	19	26.4	4	5.6	10	27.8	1	2.8	9	25	3	8.3
Constipation	22	30.6	6	8.3	10	27.8	5	13.9	12	33.3	1	2.8
Fatigue	47	65.3	2	2.8	25	69.4	1	2.8	22	61.1	1	2.8
Peripheral neuropathy	55	76.4	4	5.6	27	75.0	1	2.8	28	77.8	3	8.3
Pneumonitis	8	11.1	4	5.6	5	13.9	3	8.3	3	8.3	1	2.8
Alopecia	68	94.4			34	94.4			34	94.4		
Rash	28	38.9			15	41.7			13	36.1		
ALT	25	34.7			17	47.2			8	22.2		
Leukopenia	65	90.3	27	37.5	32	88.9	13	36.1	33	91.7	14	38.9
Neutropenia	60	83.3	22	30.6	29	80.6	13	36.1	31	86.1	9	25.0
Anemia	59	81.9	9	12.5	29	80.6	3	8.3	30	83.3	6	16.7
Thrombocytopenia	7	9.7			6	16.7			1	2.8		

ALT alanine aminotransferase

Table 3 Response according to WHO and RECIST criteria

Criteria	Study	Number of patients						RR (%)	95% CI
		Assessable patients	CR	PR	NC/SD	PD	NE		
WHO	Total	72	5	20	23	18	6	34.7	23.9, 46.9
	Early	36	2	10	9	11	4	33.3	18.6, 51.0
	Late	36	3	10	14	7	2	36.1	20.8, 53.8
RECIST	Total	69	4	16	33	9	7	29.0	18.7, 41.2
	Early	35	2	7	15	7	4	25.7	12.5, 43.3
	Late	34	2	9	18	2	3	32.4	17.4, 50.5

CR complete response, *PR* partial response, *NC* no change, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *RR* response rate, *CI* confidence interval, *WHO* World Health Organization, *RECIST* response evaluation criteria in solid tumors

(95% CI: 9.9, 16.9 months), respectively. As 11 patients (15.3%) had non-squamous cell carcinomas histology, which included 4 with adenoid cystic carcinoma and 7 with either mucoepidermoid tumor, adenocarcinoma, poorly differentiated carcinoma, acinar cell carcinoma, carcinoma, large cell carcinoma, or undifferentiated carcinoma, MST was also determined excluding these patients. MST was 13.4 months in the 61 patients with squamous cell carcinomas and 11.7 months in the 45 patients with squamous cell carcinomas of the oral cavity, paranasal cavity, oropharynx, hypopharynx, and larynx cancer. In the 23 patients who had received prior platinum-based chemotherapy for recurrent/metastatic disease, median TTP and MST were 3.2 months (95% CI: 2.5, 6.7 months) and 11.4 months (95% CI: 7.4, 19.4 months), respectively.

Discussion

Here, we conducted early and late phase II trials of weekly paclitaxel in patients with recurrent or metastatic HNC. Results demonstrated comparable safety and efficacy between the two trials. Further, the combined RR of the two trials was comparable to those previously reported in studies of tri-weekly paclitaxel in patients with advanced or recurrent HNC [6, 27]. All adverse events that occurred in the two trials were manageable, and no treatment-related deaths were observed. Although most patients had received prior chemotherapy, MST was 14.3 months, which was superior to that of previous studies in first-line patients with recurrent or metastatic HNC.

Of interest, MST in the 64 patients excluding those with nasopharyngeal cancer and in the 23 who had received

Table 4 Response rates according to patient characteristics (WHO)

Characteristic	Number of patients					RR (%)
	CR	PR	NC	PD	NE	
Sex						
Male	3	16	19	14	4	33.9
Female	2	4	4	4	2	37.5
Age (Years)						
<65	4	12	12	16	6	32.0
≥65	1	8	11	2		40.9
Histopathological diagnosis						
Squamous cell carcinoma	3	16	21	16	5	31.1
Adenoid cystic carcinoma		1	1	2		25.0
Others	2	3	1		1	71.4
Primary lesion						
Oral cavity		4	1	2	1	50.0
Nasal cavity				1		0
Paranasal cavity	1	2	4	1		37.5
Maxillary sinus				1		0
Nasopharynx	1	3	3		1	50.0
Oropharynx	1	4	3	4		41.7
Hypopharynx	1	4	8	3	2	27.8
Larynx		1	2	2	1	16.7
Salivary gland	1	2	1	2	1	42.9
Tympanum			1			0
External auditory canal				2		0
Prior radiotherapy						
None		7	1	3	1	58.3
Radiotherapy*	5	13	22	15	5	30.0
Prior chemotherapy						
None	1	3	3	2	1	40.0
Cisplatin-based chemotherapy	4	14	17	16	4	32.7
Others		3	3		1	42.9

CR complete response, *PR* partial response, *NC* no change, *PD* progressive disease, *NE* not evaluable, *RR* response rate, *WHO* World Health Organization

* Including adjuvant therapy, neoadjuvant therapy, and chemoradiotherapy

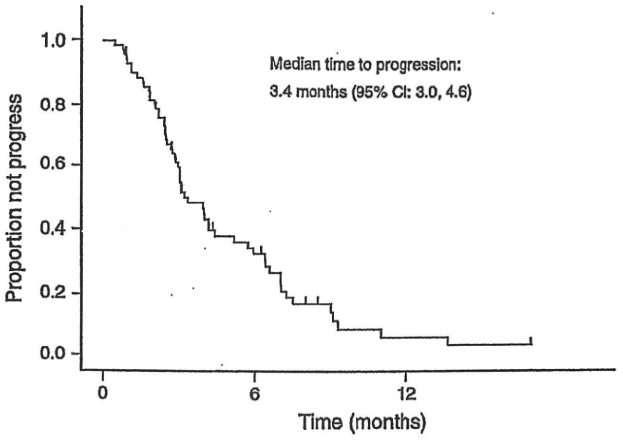

Fig. 1 Combined time to progression from the early and late phase II studies. The median time to progression was 3.4 months (95% CI: 3.0, 4.6 months)

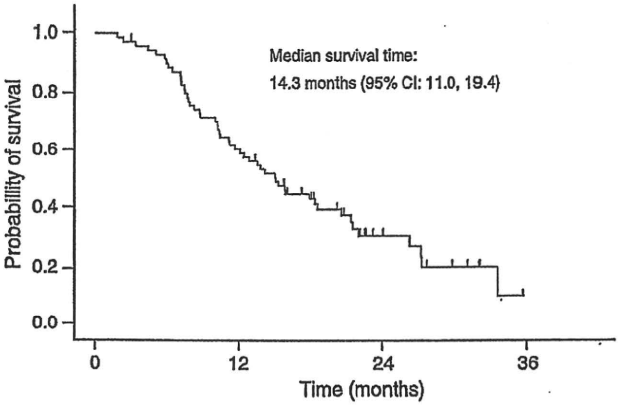

Fig. 2 Combined overall survival from the early and late phase II studies. The median follow-up time of patients for overall survival was 13.8 months, with a median overall survival time of 14.3 months (95% CI: 11.0, 19.4 months)

prior platinum-based chemotherapy for recurrent/metastatic disease was 13.0 and 11.4 months, respectively. Allowing for the fact that this was a nonrandomized trial with a relatively small number of patients, these results are nevertheless better than those in the previous studies, particularly in showing that weekly paclitaxel was active in the treatment of HNC whether patients had received prior platinum-based chemotherapy or not.

Recently, the addition of cetuximab to platinum-based chemotherapy was shown to significantly prolong overall survival without exacerbating chemotherapy-associated toxicity or quality of life in patients with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN) [10]. Furthermore, the addition of cetuximab to paclitaxel was also shown to exert promising activity in a first-line setting of a phase II trial, which had an RR of 71% and a complete response rate of 20%. Weekly paclitaxel might therefore be a good alternative to platinum-based chemotherapy for first-line patients with recurrent or metastatic SCCHN.

Treatment options for patients with recurrent or metastatic HNC who are refractory to platinum-based chemotherapy are limited. Several second-line chemotherapy regimens with cytotoxic agents, including methotrexate, vinorelbine, bleomycin, docetaxel, and S-1, have been investigated in the treatment of patients with recurrent or metastatic HNC after previous platinum-based chemotherapy [7, 11–14, 36]. Response rates and MST in these studies were 10–46.2% and less than 5 months, respectively, and it has accordingly not been possible to draw definitive conclusions on their clinical benefit.

Recently, a single institutional prospective study of weekly paclitaxel (80 mg/m^2, weekly, 6 consecutive weeks) in SCCHN patients in whom platinum-based chemotherapy failed demonstrated a response rate of 43.3% and MST of 8.5 months [9]. Although this rate is superior to that of the present study, the study was conducted at a single institution and had no independent safety and efficacy assessment committee, while our study was a multicenter trial with independent safety and efficacy assessment committees. Further, our present study demonstrated a better duration of response and survival, which might be associated with the higher dose of paclitaxel in the present study.

A combined analysis of second-line use of cetuximab with or without platinum-based chemotherapy for patients with recurrent/metastatic SCCHN in whom platinum-based chemotherapy failed concluded that cetuximab would be effective as monotherapy and could be considered a therapeutic option [29]. However, the response rate, median TTP and MST of cetuximab alone in these patients were 13%, 2.3, and 5.9 months, respectively, indicating the need for further optimization of treatment options.

Although the number of patients who had previously received platinum-based chemotherapy for recurrent/metastatic disease in the present study was small, weekly paclitaxel showed a superior response rate and survival to that of previously reported agents and may therefore also be promising in second-line treatment following cisplatin-based regimens. Recently, weekly taxane-based chemotherapy was shown to exhibit promising activity as an induction chemotherapy in the primary therapy setting [17, 25, 33], suggesting that this dose-dense strategy may be particularly applicable to sequential treatment programs for HNC.

Long-term administration of weekly paclitaxel increases the incidence and severity of peripheral neuropathy, which often reduces quality of life. In our present patients who experienced peripheral neuropathy, 14.5% recovered and 7.3% remitted, while 78.2% failed to recover by the end of the protocol. Such sustained peripheral neuropathy may be limiting for patients receiving longer-term palliative therapy. Several studies have investigated anti-neuropathy drugs, including amifostine, gabapentin, and vitamin E, but all failed to demonstrate any benefit for these patients [2, 8, 18, 19, 21, 23]. The development of effective anti-neuropathy drugs is desirable.

Several limitations of the present study warrant mention. First, subjects included eight patients with nasopharyngeal cancer, which is considered to carry a better prognosis than other HNCs. Second, subjects included chemo-naive patients and patients who had not been confirmed to be refractory to platinum-based chemotherapy. Third, the present trials were nonrandomized, and differences in patient populations due to selection bias may have influenced outcomes and toxicity rates and thereby limit comparisons between studies. Fourth, the study included a range of histological subtypes. In other words, the subjects represented a markedly heterogeneous population.

In summary, this study demonstrated that weekly paclitaxel has promising activity with acceptable toxicity in the treatment of recurrent or metastatic HNC. Paclitaxel may be a good treatment option for recurrent or metastatic HNC.

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Identification of Stem-like Cells in Head and Neck Cancer Cell Lines

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Abstract. *This study investigated the existence of stem-like cells in established head and neck squamous cell carcinoma (HNSCC) lines, HSC3 and HSC4. Flow cytometric analysis confirmed the presence of side population (SP) cells excluding Hoechst 33342 in HSC4 cells (0.37±0.06%) but not HSC3 cells in a reserpine-sensitive manner. After sorting, the SP cells generated both SP and main population (MP) cells in culture while MP cells generated MP cells only. Higher expression of stem cell markers was detected in SP than in MP cells. These results suggest that cancer stem-like cells exist in head and neck squamous cell carcinoma.*

Head and neck squamous cell carcinomas (HNSCC) are the sixth most prevalent type of malignancy worldwide (1). The most common type of HNSCC is oral squamous cell carcimoma (OCC). The overall five-year survival rate in patients with HNSCC is still lower than 50% (2, 3). HNSCC frequently shows local recurrence after the initial surgical or radiological treatment at the primary site, and even after complete resection (2, 3).

Recent data have demonstrated that tumours contain a small subpopulation of cells called cancer stem-like cells (CSCs), which are responsible for tumour maintenance and metastasis (4, 5). CSCs have the ability to self-renew and are responsible for tumorigenesis, progression, metastasis, and relapse after treatments (6-8). Some groups have demonstrated that in HNSCC a small population of $CD44^+$ cancer cells obtained from fresh tumour tissue (9, 10, 11) or permanent cell lines (12, 13) behave like CSCs and give rise to new tumours (9).

Side population (SP) cells, characterized by the efficient efflux of Hoechst 33342 dye, are thought to be the enriched in stem cells in many normal tissues (14-16) as well as in cancer (17, 18). SP cells express various adenosine triphosphate-binding cassette (ABC) transporter family members that are responsible for drug resistance, including ABCG2 (BRCP1) (19-21). Previous studies show that CSCs of HNSCC can be identified by SP phenotype from established cancer cell lines (22-25).

In this study, the existence of SP cells in HNSCC cell line was investigated by fluorescence-activated cell sorting (FACS) analysis and their characteristics identified.

Materials and Methods

Cell lines and cell culture. The HNSCC cell lines used were HSC3 and HSC4, from JCRB cell bank (Osaka, Japan). The cells were cultured in MEM (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (FBS; JRH Biosciences, Lenexa, Kansas, USA), 1% antibiotic-antimycotic mixture stock solution (100×) (nacalai tesque, Kyoto, Japan), and maintained at 37°C in a humidified 5% CO_2/95% air atmosphere.

SP analysis and cell sorting. The single-cell suspension (10^6 cells/ml) was incubated in Hank's solution containing 3 μg/ml Hoechst 33342 (Sigma-Aldrich, Saint Louis, Missouri, USA) at 37°C for 60 min with intermittent mixing. The control cells were incubated in the presence of 20 μg/ml of reserpine (Daiichi-sankyo, Tokyo, Japan). After incubation, cells were washed in Hank's solution. Propidium iodine (1 μg/ml) was added to discriminate dead cells. Analysis and sorting were performed using Beckman Coulter flow cytometry. SP and main population (MP) cells (1×10^6 cells separately) were cultured in MEM with 10% foetal calf serum (FCS) for 5-days. Each cell was stained with Hoechst 33342 and analysed using EPICS ALTRA HyPerSort (Beckman Coulter, Inc. Fullerton, CA, USA). Each experiment was performed at least 3 times.

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Key Words: Cancer stem cells, side population, head and neck squamous carcinoma.