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Phase II study of trastuzumab in combination with S-1 plus cisplatin in HER2-positive gastric cancer (HERBIS-1)

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Background: S-1, an oral fluoropyrimidine, plus cisplatin (SP) is a standard regimen for advanced gastric cancer (AGC) in East Asia. To date, no studies have evaluated the efficacy and safety of trastuzumab combined with SP in patients with human epidermal growth factor receptor type 2 (HER2)-positive AGC.

Methods: Patients with HER2-positive AGC received S-1 (80–120 mg per day) orally on days 1–14, cisplatin (60 mg m^{-2}) intravenously on day 1, and trastuzumab (course 1, 8 mg kg^{-1}; course 2 onward, 6 mg kg^{-1}) intravenously on day 1 of a 21-day cycle. The primary end point was response rate (RR); secondary end points included overall survival (OS), progression-free survival (PFS), time to treatment failure (TTF), and adverse events.

Results: A total of 56 patients were enrolled. In the full analysis set of 53 patients, the confirmed RR was 68% (95% confidence interval (CI) = 54–80%), and the disease control rate was 94% (95% CI = 84–99%). Median OS, PFS, and TTF were estimated as 16.0, 7.8, and 5.7 months, respectively. Major grade 3 or 4 adverse events included neutropaenia (36%), anorexia (23%), and anaemia (15%).

Conclusions: Trastuzumab in combination with SP showed promising antitumour activity and manageable toxic effects in patients with HER2-positive AGC.

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Gastric cancer is the second leading cause of cancer deaths worldwide (Ferlay *et al*, 2010). A global standard regimen for to treat advanced gastric cancer (AGC) has not been established (Macdonald *et al*, 2001; Cunningham *et al*, 2008). In Western countries, regimens containing a fluoropyrimidine (fluorouracil or an oral preparation) plus a platinum compound, and usually including docetaxel or epirubicin, have been most widely used. In East Asia, including Japan and Korea, a fluoropyrimidine plus a platinum compound has been used as standard therapy (Koizumi *et al*, 2008; Kang *et al*, 2009).

Recent studies have shed new light on the molecular mechanisms underlying the development and progression of gastric cancer. Trastuzumab is a monoclonal antibody targeting human epidermal growth factor receptor type 2 (HER2) with two antigen-specific sites that bind to the juxtamembrane portion of the extracellular domain of the HER2 receptor, thereby preventing activation of its intracellular tyrosine kinase (Hudis, 2007). The Trastuzumab for Gastric Cancer (ToGA) study, an international phase III trial comparing chemotherapy consisting of cisplatin plus capecitabine or fluorouracil *vs* trastuzumab plus chemotherapy in patients with HER2-positive AGC, demonstrated a survival benefit with the addition of trastuzumab (Bang *et al*, 2010). Currently, both the US Food and Drug Administration and the European Medicines Agency approved trastuzumab for the treatment of patients with HER2-positive AGC, and trastuzumab in combination with cisplatin plus capecitabine or fluorouracil is a standard treatment for HER2-positive AGC in the West.

S-1 is a fluoropyrimidine preparation combining tegafur, a prodrug of 5-fluorouracil (5-FU), gimeracil, and oteracil potassium in a molar ratio of 1 : 0.4 : 1. Gimeracil is a dihydropyrimidine dehydrogenase inhibitor, allowing high concentrations of 5-FU to be maintained (Shirasaka *et al*, 1996; Diasio, 1999). Two phase II studies (Sakata *et al*, 1998; Koizumi *et al*, 2000) in patients with AGC showed response rates (RRs) exceeding 40%. The S-1 Plus cisplatin versus S-1 In RCT In the treatment for Stomach cancer (SPIRITS) phase III trial established S-1 plus cisplatin (SP) as a standard first-line regimen for AGC in the East (Koizumi *et al*, 2008; Japanese Gastric Cancer Association, 2011). However, SP plus trastuzumab has not been evaluated in patients with HER2-positive AGC to date. We therefore conducted this phase II study to evaluate the efficacy and safety of SP plus trastuzumab in HER2-positive AGC.

PATIENTS AND METHODS

Patients. We enrolled patients with histologically proven unresectable or recurrent HER2-positive tumours in the stomach or gastroesophageal junction. Human epidermal growth factor receptor type 2 status of tumours was evaluated using immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH). In the IHC testing, HER2 tumour cell-membrane immunostaining was scored using a four-grade scale (0/1+/2+/3+) according to scoring scheme (ToGA score): 0, no staining or membranous reactivity in $<10\%$ of tumour cells; 1+, weak, barely perceptible membranous reactivity in $>10\%$ of tumour cells; 2+, complete or basolateral membranous reactivity either nonuniform or weak in $\geqslant 10\%$ of cells; and 3+, complete or basolateral membranous reactivity of strong intensity in $\geqslant 10\%$ of tumour cells (Hofmann *et al*, 2008; Bang *et al*, 2010). FISH analyses for HER2 status were carried out according to the manufacturer's procedure. The total numbers of HER2 and chromosome 17 signals were counted in at least 20 tumour cell nuclei in two different areas. The case with HER2/chromosome 17 ratio of $\geqslant 2.0$ was defined as FISH positive. In this study, only patients with IHC 3+, or IHC 2+ and FISH positive were eligible. Patients were required to have measurable lesions according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (Eisenhauer *et al*, 2009). Eligibility criteria also included: age between 20 and 75 years; Eastern Cooperative Oncology Group performance status score of 0 or 1; leukocyte count between 3500 and 12 000 mm^{-3}, neutrophil count $\geqslant 2000\ mm^{-3}$, hemoglobin $\geqslant 9.0\ g\,dl^{-1}$, platelet count $\geqslant 100\,000\ mm^{-3}$, serum bilirubin $<1.5\ mg\,dl^{-1}$, creatinine clearance $\geqslant 60\ ml\,min^{-1}$ calculated using the Cockcroft–Gault formula, serum creatinine $\leqslant 1.2\ mg\,dl^{-1}$, serum aspartate aminotransferase and alanine aminotransferase $<100\ IU\,l^{-1}$; and baseline left ventricular ejection fraction $\geqslant 50\%$. Patients were excluded from the study if they could not maintain sufficient oral intake, have massive ascites or pleural effusions, or had received prior chemotherapy or radiotherapy within 6 months before enrollment. The study protocol was approved by the Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG) Steering Committee and the institutional review boards of all participating hospitals. All patients provided written informed consent before enrollment. This study was registered with UMIN-CTR, UMIN000005739.

Treatment. Trastuzumab was commercially obtained in this study. Patients received cisplatin ($60\ mg\,m^{-2}$) plus trastuzumab (course 1, $8\ mg\,kg^{-1}$; course 2 onward, $6\ mg\,kg^{-1}$) intravenously on day 1 and oral S-1 twice daily at a dose based on body surface area ($<1.25\ m^2$, 40 mg; $\geqslant 1.25$ to $<1.5\ m^2$, 50 mg; $\geqslant 1.5\ m^2$, 60 mg) on days 1–14 of a 21-day cycle.

This schedule was repeated until disease progression, development of unacceptable toxicity, or patient withdrawal of consent. If patients had a neutrophil count less than $1000\ mm^{-3}$, platelet count less than $75 \times 10^3\ mm^{-3}$, serum creatinine more than $1.2\ mg\,dl^{-1}$, infection with fever, or anorexia, diarrhoea, oral mucositis, or rash of grade 2 or higher, treatment with S-1 was suspended. In patients with febrile neutropaenia, grade 4 neutropaenia, grade 3–4 thrombocytopaenia, serum creatinine $>1.2\ mg\,dl^{-1}$, or grade 3–4 diarrhoea, oral mucositis, or rash, doses of S-1 and cisplatin were reduced starting from the next cycle. In patients who had grade 3–4 vomiting or anorexia because of cisplatin, the dose of cisplatin was reduced. If heart failure or severe infusion reactions occurred, treatment with trastuzumab was discontinued.

Evaluations. The primary end point was RR. The secondary end points were overall survival (OS), progression-free survival (PFS), time to treatment failure (TTF), and adverse events. Tumours were assessed every 6 weeks until disease progression, and objective responses were evaluated according to the RECIST guidelines (version 1.1). For complete response (CR) or partial response (PR), confirmation 4 weeks after initial evaluation was necessary. An independent review committee assessed responses in all patients. OS was defined as the time from the date of enrollment to the date of death from any cause. PFS was defined as the time from the date of enrollment to the date of disease progression or death from any cause. TTF was defined as the time from the date of enrollment to the date when the treating physician decided to discontinue treatment for any reason. Physical examination and blood test were mandatory before each course, and left ventricular ejection fraction was assessed every 3 month during treatment. Adverse events were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Statistical analysis. The required sample size was estimated based on a threshold RR of 35% and an expected RR of 50%, 80% power, and an alpha value of 0.1 (one-sided) using the binomial test. Given 2% of ineligible patients, the target sample size was determined to be at least 50 patients. Efficacy was evaluated in all patients who received at least one dose of the study treatment.

We used the Kaplan–Meier method to estimate survival curves and Greenwood's formula to calculate 95% confidence intervals (CIs) for survival rates. Statistical analyses were conducted with R, version 3.0.1.

RESULTS

Patient background. Between July 2011 and May 2012, a total of 56 patients were enrolled from 29 hospitals in Japan. Two patients were ineligible because of inadequate renal function or the absence of measurable lesions. The characteristics of the 54 eligible patients are listed in Table 1. The median age was 66 years (range = 34–75 years). Two-thirds of patients had differentiated adenocarcinoma. Only three patients (6%) had recurrent disease; the others had unresectable lesions. The most frequent sites of metastasis were the lymph nodes (81%), followed by the liver (59%). The proportions of IHC 3+ and IHC 2+/FISH-positive tumours were 83% and 17%, respectively.

Table 1. Eligible patient characteristics

Characteristic	*n* = 54
Age, years	
Median	66
Range	34–75
Sex	
Male	42 (78%)
Female	12 (22%)
Performance status	
0	42 (78%)
1	12 (22%)
Histological type	
Differentiated	36 (67%)
Undifferentiated	18 (33%)
Previous gastrectomy	
No	45 (83%)
Yes	9 (17%)
Unresectable/recurrent	
Unresectable	51 (94%)
Recurrent with adjuvant chemotherapy	2 (4%)
Recurrent without adjuvant chemotherapy	1 (2%)
Metastatic sites[a]	
Lymph nodes	44 (81%)
Liver	32 (59%)
Lung	5 (9%)
Peritoneum	5 (9%)
Bone	2 (4%)
Other	1 (2%)
HER2 status	
IHC 3+	45 (83%)
IHC 2+/FISH positive	9 (17%)

Abbreviations: FISH = fluorescence *in situ* hybridization; HER2 = human epidermal growth factor receptor type 2; IHC = immunohistochemistry.
[a]Some patients had multiple metastatic sites.

Efficacy. Of the 54 eligible patients, 1 patient did not receive any treatment per protocol because of a decrease in serum hemoglobin levels after study enrollment. Efficacy and safety analyses were therefore conducted in the full analysis set of the remaining 53 patients.

The median number of cycles was 6 (range = 1–27), and the median relative dose intensity for S-1, cisplatin, and trastuzumab was 76%, 83%, and 96%, respectively. At the time of analysis (August 2013), 51 patients had discontinued treatment. The main reason for discontinuation was progressive disease (31 patients), followed by adverse events (16 patients). Four patients underwent surgery because of a prominent response.

The confirmed RR based on RECIST (version 1.1) was 68% (95% CI = 54–80%; 80% CI = 58–76%; Table 2), so the null hypothesis for the primary end point (RR $\leqslant$35%) was rejected ($P<0.001$). The confirmed RRs in the differentiated type cases ($n=35$) and the undifferentiated type cases ($n=18$) were 69% (95% CI = 51–83%) and 67% (95% CI = 41–87%), respectively. Among 36 patients with CR or PR, the median time to response and duration of response were 41 days (range = 33–91 days) and 208 days (range = 42–630 days), respectively. The disease control rate, that is, the proportion of patients who had a CR, PR, or stable disease, was 94% (95% CI = 84–99%). Two patients (4%) had a CR. A waterfall plot of the confirmed best overall response for each patient is shown in Figure 1.

The median duration of follow-up at the time of analysis (August 2013) for the 53 patients was 13.5 months. The median OS was 16.0 months (95% CI = 13.3–not applicable), and the 1-year OS rate was 67.9% (95% CI = 56.5–81.7%; Figure 2). The median PFS was 7.8 months (95% CI = 6.0–8.8 months), and the 1-year PFS rate was 17.0% (95% CI = 9.4–30.8%; Figure 2). The median TTF was 5.7 months (95% CI = 4.2–7.1 months), and the 1-year TTF rate was 5.1% (95% CI = 1.4–18.6%).

Safety. All adverse events that occurred in three or more patients are shown in Table 3. Among the haematological adverse events, the proportions of grade 3–4 neutropaenia and anaemia were 36% and 15%, respectively. The most frequent common non-haematological toxicity was anorexia (any grade, 79%; grade 3–4, 23%). Except for anorexia, there were no grade 3 or 4 toxicities that occurred in more than 10% of patients. Creatinine was elevated in 24 of 53 patients (45%). Grade 2 infusion-related reactions occurred in three patients (6%). Heart failure did not occur in any patients.

There was one treatment-related death attributable to myelosuppression. This patient was judged as an ineligible case afterwards, because creatinine clearance before enrollment was 47.4 ml min^{-1}. Furthermore, S-1 administration continued despite a serum creatinine level of 2.31 mg dl^{-1} on day 7. Renal dysfunction led to myelosuppression that progressed to death. Upon review of the patient's records, the data and safety

Table 2. Objective response with confirmation based on RECIST

Total	*n* = 53
Complete response	2 (4%)
Partial response	34 (64%)
Stable disease	14 (26%)
Progressive disease	3 (6%)
Response rate (95% confidence interval)	68% (54–80%)
Disease control rate (95% confidence interval)	94% (84–99%)

Abbreviation: RECIST = Response Evaluation Criteria in Solid Tumor.

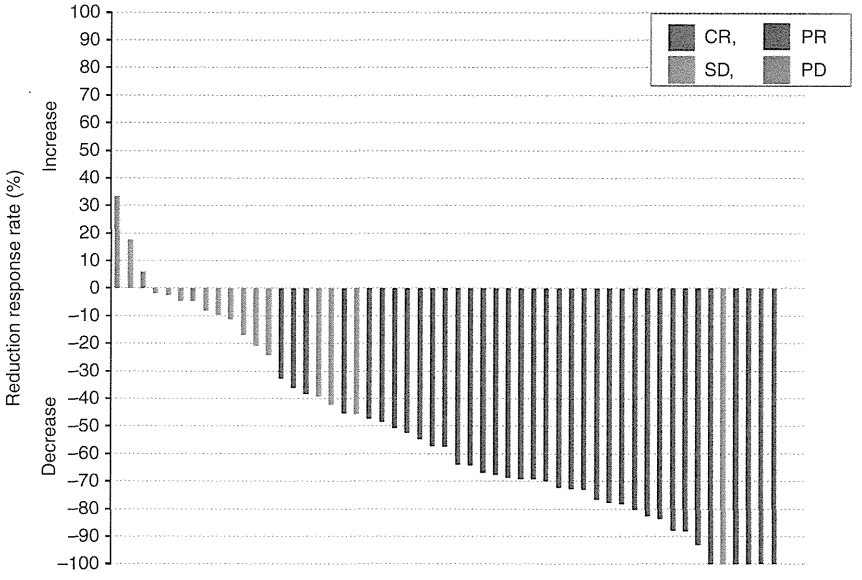

Figure 1. Waterfall plot of confirmed best overall response.

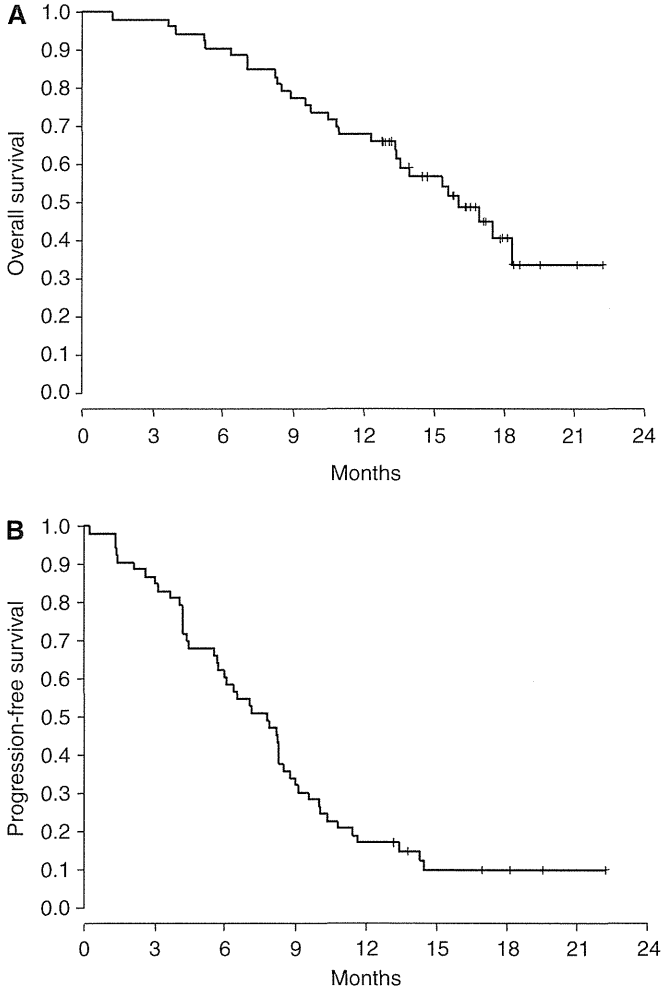

Figure 2. (**A**) The Kaplan–Meier overall survival and (**B**) progression-free survival.

monitoring committee determined that the patient died from critical deviations from the eligibility criteria and treatment protocol.

DISCUSSION

This multicenter phase II study is the first clinical trial reporting the efficacy and safety of SP plus trastuzumab in patients with HER2-positive AGC. We obtained a much higher RR (68%) than expected. The toxicity profile of our regimen was tolerable, and the incidence of grade 3–4 adverse events were similar to those of the SP regimen in the SPIRITS study (Koizumi *et al*, 2008). These results suggest that SP plus trastuzumab is a potential new treatment option for patients with HER2-positive AGC.

The ToGA study demonstrated that trastuzumab in combination with cisplatin plus capecitabine or fluorouracil was superior to cisplatin plus capecitabine or fluorouracil alone (Bang *et al*, 2010). The RR was 35% in the chemotherapy group and 47% in the trastuzumab plus chemotherapy group. In the aforementioned phase II study of a 3-week cycle of SP, the RR was 48%, compared with 68% in the present study, suggesting that trastuzumab considerably enhanced the effectiveness of chemotherapy, which is consistent with the results of the ToGA study. In addition, the median OS and PFS in our study were 16.0 and 7.8 months, respectively, whereas the subgroup of Japanese patients in the trastuzumab arm of the ToGA study had a median OS and PFS of 15.9 and 6.2 months, respectively (Sawaki *et al*, 2012). Although these results must be interpreted with caution because of the differences between the ToGA study and our study in terms of patient characteristics, especially histologic type, the proportion of patients with HER2 IHC 3+ tumours, and exclusion of patients with performance status $\geqslant 2$, trastuzumab may be a good addition to a S-1-based regimen. Experimental studies have reported that trastuzumab induces downregulation of thymidylate synthase expression. This mechanism has been implicated in the synergistic antitumour effect of S-1 plus trastuzumab against gastric cancer cell lines that overexpress HER2 (Tanizaki *et al*, 2010). Capecitabine and S-1 are both 5-FU derivatives, but were developed based on different concepts. Further studies of biomarkers and other predictors of outcomes are necessary to optimise the use of these drugs.

During the planning phase of this trial, a 5-week cycle of SP therapy was the mainstay of chemotherapy for AGC in Japan, based on the results of the SPIRITS study (Koizumi *et al*, 2008). As a molecular-targeted agent was combined with SP, the development of a 3-week cycle was planned. Results of phase II studies of a 3-week regimen of SP have been reported in gastric

Table 3. Adverse events ($n = 53$)

	Grade					
Event	1	2	3	4	Any (%)	Grade 3–4 (%)
Leukopaenia	17	18	3	1	74	8
Neutropaenia	8	5	14	5	60	36
Febrile neutropaenia	0	0	1	1	4	4
Anaemia	5	22	6	2	66	15
Thrombocytopaenia	20	6	0	0	49	0
Anorexia	15	15	12	0	79	23
Fatigue	18	14	2	0	64	4
Nausea	20	12	1	0	62	2
Hypoalbuminaemia	14	6	5	0	47	9
Hypertension	9	12	1	0	42	2
Creatinine increased	21	0	3	0	45	6
Diarrhoea	10	7	4	0	40	8
Oral mucositis	10	6	1	0	32	2
Skin rash	12	1	0	0	25	0
Vomiting	7	3	3	0	25	6
ALT increased	11	2	0	0	25	0
Constipation	7	4	0	0	21	0
Dysgeusia	7	3	0	0	19	0
AST increased	9	0	0	0	17	0
Blood bilirubin increased	6	2	0	0	15	0
Edema	6	2	0	0	15	0
Peripheral sensory neuropathy	1	5	0	0	11	0
Epistaxis	3	1	0	0	8	0
Hiccups	4	0	0	0	8	0
Fever	2	2	0	0	8	0
Infusion-related reaction	0	3	0	0	6	0
Alopecia	2	1	0	0	6	0
Abdominal pain	1	2	0	0	6	0
Skin hyperpigmentation	2	1	0	0	6	0

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase.

cancer and lung cancer (Lee *et al*, 2008; Choi *et al*, 2010; Kubota *et al*, 2010). Recently, a phase III trial comparing the standard 5-week cycle of SP with a 3-week cycle of SP was conducted in patients with AGC. This trial showed that the median PFSs in the 3-week and 5-week cycle groups were 5.5 and 4.9 months, respectively, and it concluded that a 3-week cycle of SP was superior to a 5-week cycle of SP in terms of PFS ($P = 0.042$) (Ryu *et al*, 2013). We therefore expected that a 3-week regimen of SP plus trastuzumab would be more effective than a 5-week regimen of SP plus trastuzumab. Although the dose intensity of cisplatin (20 mg m^{-2} per week) in a 3-week SP regimen was 25% lower than that (26.7 mg m^{-2} per week) in the ToGA study regimen, the RR (48%) of 3-week SP regimen was higher than that (35%) of the ToGA regimen. Thus, we considered that the dose (60 mg m^{-2}) of cisplatin was adequate in this 3-week SP regimen.

In this study, we limited subjects to patients with measurable lesions assessable according to RECIST guidelines (version 1.1). In clinical practice, however, many patients with gastric cancer have no measurable lesions, such as those with peritoneal metastasis. We are therefore conducting another phase II study in patients who have HER2-positive AGC without measurable lesions (HERBIS-1B; UMIN000007941) to confirm the usefulness of this regimen in this subgroup.

In conclusion, although this was not a randomised controlled study, our results suggest that SP plus trastuzumab has a good toxicity profile and promising efficacy, justifying the further study of regimens that contain SP and trastuzumab.

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CONFLICT OF INTEREST

Y Kurokawa, H Imamura, Y Komatsu, Y Doki, and T Tsujinaka received speaker honoraria from Taiho Pharmaceutical. Y Komatsu and Y Doki received unrestricted research grant from Taiho Pharmaceutical. The remaining authors declare no conflict of interest.

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ORIGINAL ARTICLE

Double-blind, placebo-controlled, randomized phase II study of TJ-14 (hangeshashinto) for gastric cancer chemotherapy-induced oral mucositis

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Abstract

Background Hangeshashinto (TJ-14, a Kampo medicine), which reduces the level of prostaglandin E2 and affects the cyclooxygenase activity, alleviates chemotherapy-induced oral mucositis (COM). We conducted a randomized comparative trial to investigate whether TJ-14 prevents and controls COM in patients with gastric cancer.

Methods We randomly assigned patients with gastric cancer who developed moderate-to-severe oral mucositis (CTCAE v4.0 grade ≧1) during any cycle of chemotherapy to receive either TJ-14 or a placebo as a double-blind trial. The patients received a placebo or TJ-14 for 2–6 weeks according to the chemotherapy regimen from the beginning of the next course of chemotherapy. The primary end point was the incidence of grade ≧2 oral mucositis in the protocol treatment course, and the secondary end points were the time to disappearance of oral mucositis and the incidence of adverse events.

Results Following the key opening of the blinding protocol, we analyzed 91 eligible patients (TJ-14: 45, placebo: 46) using a "per protocol set" analysis. The incidence of ≧grade 2 COM was 40.0 % in the TJ-14 group and 41.3 % in the placebo group ($p = 0.588$). The median duration of ≧grade 2 COM was 14 days in the TJ-14 group and 16 days in the placebo group ($p = 0.894$). Meanwhile, the median duration of any grade of COM was 9 days in the TJ-14 group and 17 days in the placebo group among the patients who developed grade 1 symptoms during the screening cycle [hazard ratio 0.60; 95 % CI (0.23–1.59), $p = 0.290$].

Conclusions Although TJ-14 treatment did not reduce the incidence of ≥2 COM in the patients who developed mucositis during chemotherapy for gastric cancer, a trend was observed in which TJ-14 reduced the risk of COM in the patients who developed grade 1 COM during the screening cycle. Further, phase III studies with a larger sample size are needed to clarify the protective effects of TJ-14 for COM.

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Keywords Oral mucositis · Hangeshashinto (TJ-14) · Chemotherapy · Gastric cancer

Introduction

Gastric cancer is the second most frequent cancer-related cause of death after lung cancer [1]. Chemotherapy is one of the most important modalities for treating advanced gastric cancer as well as curatively resected cancers in the adjuvant setting. Numerous chemotherapy regimens have been used in cases of operable or inoperable gastric cancer [2–5]. Although several studies have shown that chemotherapy improves and prolongs survival, it often causes severe toxicity, seriously compromising the patient's quality of life and precluding the continuation of the treatment.

Oral mucositis is a common toxicity associated with cytotoxic chemotherapy used in the gastric cancer treatment. In pivotal phase III trials of chemotherapy for gastric cancer, the incidence of all grades of chemotherapy-induced oral mucositis (COM) was observed to be 6.3–32 % [4–8]. COM results in severe discomfort, impairing the patient's ability to eat, swallow, and talk, and has an indirect effect on tumor outcomes, as its presence often necessitates the unfavorable modification of anticancer therapy, such as breaks in the administration of chemotherapy or dose reduction in the chemotherapy regimen [9–11]. One factor associated with COM exacerbation is the activation of the cyclooxygenase pathway, which mediates ulcer formation and pain via the upregulation of pro-inflammatory prostaglandins. Indeed, Richard et al. demonstrated, after having enlisted 20 patients treated with chemotherapy drugs and performing a biopsy of the oral mucosa in each case, a statistically significant increase in the number of endothelial cells in the oral mucosa with nuclear factor-kappa B (NF-κB) and cyclooxygenase 2 (COX-2) expressions in the postchemotherapy treatment period compared to that observed in the pretreatment period. The expression of COX-2 in these cells represents the initial sign of the inflammatory cascade that determines the production of prostaglandins and further tissue damage. COX-2 is also upregulated by NF-κB, which plays an important role in the inflammatory process [12]. COM invariably requires treatment with systemic analgesics, adjunctive medications, physical therapy, and psychological therapy in addition to oral care [13]. Treatment guidelines developed by the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology have been published; however, they also highlighted the need for a higher level of evidence [14]. Although a range of interventions have been developed to prevent and treat COM, a more rational approach is warranted [11].

Hangeshashinto (TJ-14) is a traditional Japanese medicine containing 7 herbal crude drugs. Seven herbal crude drugs are as follows; Pinelliae tuber, Scutellariae Radix, Glycyrrhizae Radix, Zizyphi Fructus, Ginseng Radix, Zingiberis Processum rhizoma, and Coptidis rhizome [15–17]. TJ-14 is prescribed in Japan to treat inflammatory diarrhea, gastritis, and stomatitis. Recently, Kono et al. [18] found that TJ-14 was effective as a gargle therapy for the treatment of COM in a pilot clinical study and a randomized, placebo-controlled clinical trial. TJ-14 has been demonstrated to directly inhibit PGE2 production in human gingival fibroblasts and reduce the PGE2 content in the colon in several animal models of diarrhea using anticancer drugs, cholera toxin, or castor oil, resulting in the amelioration of inflammatory damage [19–22]. It has also been reported that some ingredients of TJ-14 inhibit PGE2 production and/or the COX-2 expression [23–32]. Phenylpropanoids, such as [6]-shogaol and [6]-gingerol, flavonoids, such as wogonin, baicalein, and baicalin, and isoquinoline alkaloids, such as berberine, are well established to possess an anti-PGE2 activity via various particular mechanisms.

Considering these clinical and biochemical study findings, in the present study, the efficacy of TJ-14 in the prevention and/or treatment of COM was investigated in a randomized, double-blind, placebo-controlled clinical trial of patients receiving chemotherapy for gastric cancer.

Materials and methods

Study design

A prospective, multi-institutional, randomized, double-blind, placebo-controlled phase II trial was performed in patients receiving chemotherapy for gastric cancer in Japan. Patients who developed CTCAE v4.0 ≧grade 1 oral mucositis during the screening cycle of chemotherapy were considered eligible for inclusion in this study. The eligible patients were centrally randomized to receive either TJ-14 or a placebo during their next cycle of chemotherapy. The patients were stratified according to age, chemotherapy regimen, institution, and previous treatment for oral mucositis before randomization in a 1:1 ratio. A specially made and prepared matched placebo was utilized to confirm blinding.

The primary objective of this study was to determine the efficacy and safety of TJ-14 compared with the placebo. The primary end point was the incidence of ≥grade 2 oral mucositis, and the worst oral mucositis grade observed throughout the protocol therapy was assessed. As for severity, the worst grade observed on the day of the medication was evaluated instead of the mean circadian change. The secondary end points were the time to disappearance of oral mucositis and the incidence of adverse events.

Ethics

The study data and informed consent were obtained in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Review Board of each institution. The institutions where ethics was obtained were as follows: Kanagawa Cancer Center, Osaka General Medical Center, Chiba Cancer Center, Hiroshima University, Kinki University, Teikyo University, Toyonaka Municipal Hospital, Hiroshima City Asa Hospital, Prefectural Aichi Hospital, Kochi University, Minoh City Hospital, National Hospital Organization Nagoya Medical Center, Shizuoka General Hospital, Nagoya City University, and Osaka-Kita-Teishin Hospital. All patients were given a written explanation of the study protocol and provided their written informed consent before participating.

Inclusion and exclusion criteria

Patients 20 years of age or older who were undergoing chemotherapy for gastric cancer were considered eligible for this study. Patients who developed moderate-to-severe oral mucositis (CTCAE v4.0 grade $\geqq 1$) during any cycle of chemotherapy (S-1, paclitaxel, irinotecan, cisplatin, etc.) were asked to be enrolled in the study. All participants were required to have a "good" performance status (i.e., scores of 0 or 1 on the Eastern Cooperative Oncology Group performance status scale). Patients with any of the following characteristics were not eligible for the study: use of Kampo medicine within 2 weeks before registration and a history of severe hypersensitivity (allergy) to any medicine containing antiphlogistics, analgesics, opioids, or steroids. Patients with serious constipation and pregnant or lactating females were excluded from the study. Any other medical conditions that made a patient unsuitable for inclusion in the study according to the opinion of the investigator were also considered to be exclusion criteria for this study.

Chemotherapy

Gastric cancer chemotherapy was administered according to the protocols of each treatment, and the administration of each agent was described in case report form. Patients enrolled in this study received the following chemotherapy.

Group A: S-1 monotherapy. S-1: The treatment regimen consisted of 6-week cycles in which 80 mg/m^2 per day was given for 4 weeks followed by 2-week rest for adjuvant setting and 5-week cycles in which 80 mg/m^2 per day for 3 weeks followed by 2-week rest for advanced gastric cancer patients

Group B: S-1 plus cisplatin. S-1: 80 mg/m^2 oral administration (p.o.) daily for 21 days, every 5 weeks. Cisplatin: 60 mg/m^2 intravenous drip (d.i.v.) day 8, every 5 weeks.

Group C: S-1 plus paclitaxel. S-1: 80 mg/m^2 p.o. daily on days 1–14 of 3 weeks cycle. Paclitaxel: 50 mg/m^2 d.i.v. days 1, 8 every 3 weeks.

Group D: paclitaxel. Paclitaxel: 80 mg/m^2 d.i.v. days 1, 8, 15, every 4 weeks.

Group E: S-1 plus docetaxel. S-1: 80 mg/m^2 p.o. daily on days 1–14 of 3 weeks cycle. Docetaxel: 40 mg/m^2 d.i.v. days 1 every 3 weeks.

Group F: docetaxel. Docetaxel: 60 mg/m^2 d.i.v. days 1 every 3 weeks.

Group G: CPT-11 plus cisplatin. CPT-11: 60 mg/m^2 d.i.v. days 1 every 2 weeks. Cisplatin: 30 mg/m^2 d.i.v. days 1 every 2 weeks.

Group H: CPT-11. CPT-11: 150 mg/m^2 d.i.v. days 1 every 2–3 weeks

Study drug

Both TJ-14 and the placebo were administered at a dose of 2.5 g/three times per day (for a total daily dose of 7.5 g). The placebo formulation matched the texture, flavor, and other characteristics of the active drug. The patients were advised to dissolve 2.5 g of TJ-14 or the placebo in 50 ml of drinking water and rinse their oral cavity with the solution three times daily for 10 s. The test drug was administered from the first day to final day of the protocol treatment course. After the protocol treatment course, TJ-14 was administrated for one course, as much as possible. The patients followed the oral care instructions throughout the treatment period before the next course of chemotherapy began. No other prophylactic mouthwashes or treatments for mucositis were allowed in this clinical trial.

Study assessment

The signs and symptoms of oral mucositis were assessed by the investigator during the screening cycle. The CTCAE v4.0 grading (Table 1) was used to assess the severity of oral mucositis. The time to healing of oral mucositis was defined as the period from the start date of the protocol treatment or the date of onset of oral mucositis to the date when all oral mucositis symptoms disappeared. If all oral mucositis symptoms fail to disappear within the study treatment period, the observation shall be continued until symptom disappearance. Additionally, the patients reported their own ability to eat solid foods. Safety was assessed throughout the study using physical examinations, including inspection of the oral tissue, hematology and serum chemistry laboratory tests, and adverse event reporting. Any adverse event, whether related or unrelated to the study drug, was reported with the date and time of onset,

Table 1 Severity of oral mucositis

Grade 1	Asymptomatic or mild symptoms; Intervention not indicated
Grade 2	Moderate pain; not interfering with oral intake; Modified diet indicated
Grade 3	Severe pain; Interfering with oral intake
Grade 4	Life-threatening consequence; Urgent intervention indicated
Grade 5	Death

Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0

severity, pattern, action taken, and outcome. If the adverse event had not resolved at the time the case report forms were collected, a follow-up report was provided a later date. If no follow-up report was provided, the investigator had to provide justification. All adverse events were followed until they either resolved or the investigator determined that the event was no longer clinically significant.

Statistical analysis

The eligible patients were randomly assigned on a 1:1 ratio to receive either TJ-14 or the placebo. After checking patient eligibility, randomization was carried out centrally at the data center using dynamic randomization with main prognostic factors, including the chemotherapy regimen (postoperative adjuvant chemotherapy, unresectable metastatic/recurrent lesions), presence/absence of previous treatment of oral mucositis, age (≥60 years, <60 years), and institution.

Assuming an incidence of grade 2 or worse COM of 10 % in the TJ-14 group and 35 % in the placebo group, a sample size of 42 for each group was estimated to have at least 80 % power under a significance level of two-sided 10 %. Therefore, in order to account for possible dropouts, a target sample size of 90 patients was required.

The difference in the incidence of grade 2 or worse COM between the groups and its 90 % confidence interval was calculated. Comparisons were made using the chi-squared test. The baseline characteristics were compared using the chi-squared test for categorical variables and the Wilcoxon test for continuous variables. The Kaplan–Meier method, log-rank test, and Cox proportional hazard regression model were used to assess the time to healing among the patients with COM. A hazard ratio (HR) smaller than 1 indicated that TJ-14 accelerated the healing of COM. The frequencies of adverse events were compared using Fisher's exact test. All p values were two-sided. The statistical analyses were performed using the SAS software package for Windows, release 9.3 (SAS Institute, Cary, NC).

Results

Patients

Of the patients receiving chemotherapy for gastric cancer, 91 who developed CTCAE v4.0 ≧grade 1 oral mucositis during the screening cycle and provided informed consent were randomized to either the TJ-14 ($n = 45$) or placebo ($n = 46$) group. The baseline demographics and disease characteristics of the per protocol set (PPS) population are shown in Table 2. A total of 61.5 % of the subjects were male, and 38.5 % of the subjects were female; the median age was 68 years (range 36–89 years). All patients had histologically confirmed gastric adenocarcinoma. There were no disparities between the two PPS randomized groups. The majority of patients received S-1 adjuvant (48.4 %) or S-1-based doublet (22.0 %) regimens, and the treatment groups were balanced for the chemotherapy regimen (Table 2). No patients received radiation therapy or molecular targeting agents before enrollment. No patients were enrolled in the study if there was any clinical evidence of another active oral mucosal disease at baseline.

Incidence and duration of COM

The incidence of ≧grade 2 COM was 40 % (18 patients) in the TJ-14 group and 41.3 % (19 patients) in the placebo group, and there was no significant difference between the two groups ($p = 0.588$); the primary end point was not met in this study. More, when comparing the duration of ≧grade 2 COM between the two treatment groups, there was not significantly difference (HR 0.97 (0.41–2.29) log-rank $p = 0.937$) (Fig. 1).

However, among the patients who developed Grade 1 COM during the screening cycle, the median duration of any grade of COM was 9.0 days in the TJ-14 group and 17.0 days in the placebo group [HR 0.598; 95 % CI (0.226–1.585), $p = 0.290$] (Fig. 2). Treatment with TJ-14 reduced the duration of any grade of COM compared with the placebo.

Chemotherapy treatment failure during the protocol treatment

Chemotherapy treatment failure was observed in 26.7 % (12 patients) of the subjects in the TJ-14 group and 21.7 % (10 patients) of the subjects in the placebo group. For most chemotherapy regimens, there were no significant differences with regard to the incidence of the treatment failure.

Safety

Hematological, blood biochemistry, and non-hematological toxicities were analyzed. The most commonly reported

Table 2 Patient characteristics of the TJ-14 and placebo groups

Treatment	Placebo ($N = 46$)	TJ-14 ($N = 45$)	p value
Sex			
Male	28 (60.9 %)	28 (62.2 %)	0.895
Female	18 (39.1 %)	17 (37.8 %)	
Age			
Median	67.5	68.0	0.648
Range	42.0–89.0	36.0–84.0	
PS			
0	38 (82.6 %)	39 (86.7 %)	0.855
1	5 (10.9 %)	4 (8.9 %)	
2	3 (6.5 %)	2 (4.4 %)	
Status			
Adjuvant	21 (45.7 %)	23 (51.1 %)	0.602
Advanced	25 (54.3 %)	22 (48.9 %)	
Oral care (patients)			
+	3 (6.5 %)	2 (4.4 %)	0.664
−	43 (93.5 %)	43 (95.6 %)	
Oral care (institution)			
+	11 (23.9 %)	7 (15.6 %)	0.317
−	35 (76.1 %)	38 (84.4 %)	
Chemotherapy at the time of registration			
S-1	19 (42.2 %)	25 (55.6 %)	0.490
S-1 + CDDP	3 (6.7 %)	5 (11.1 %)	
S-1 + DTX	6 (13.3 %)	1 (2.2 %)	
S-1 + PTX	2 (4.4 %)	3 (6.7 %)	
DTX	1 (2.2 %)	1 (2.2 %)	
PTX	3 (6.7 %)	2 (4.4 %)	
CPT-11 + CDDP	1 (2.2 %)	2 (4.4 %)	
CPT-11	3 (6.7 %)	1 (2.2 %)	
5-FU + CDDP	0 (0 %)	1 (2.2 %)	
other	7 (15.6 %)	4 (8.9 %)	

CDDP Cisplatin, *PTX* Paclitaxel, *DTX* Docetaxel, *5-FU* 5-fluorouracile

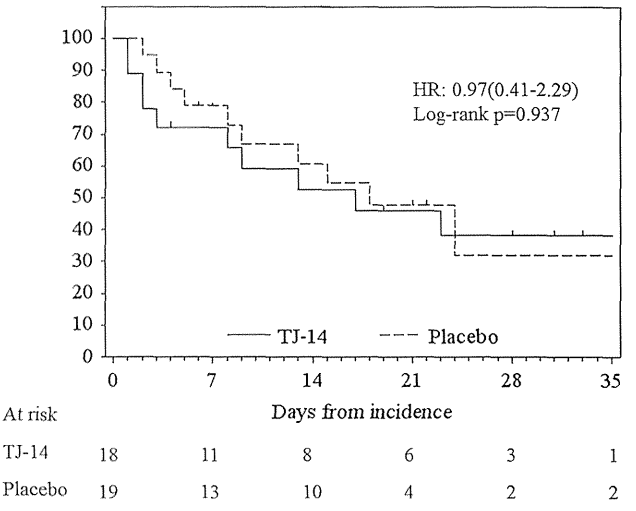

Fig. 1 Duration of ≧grade 2 chemotherapy-induced oral mucositis in the treatment group

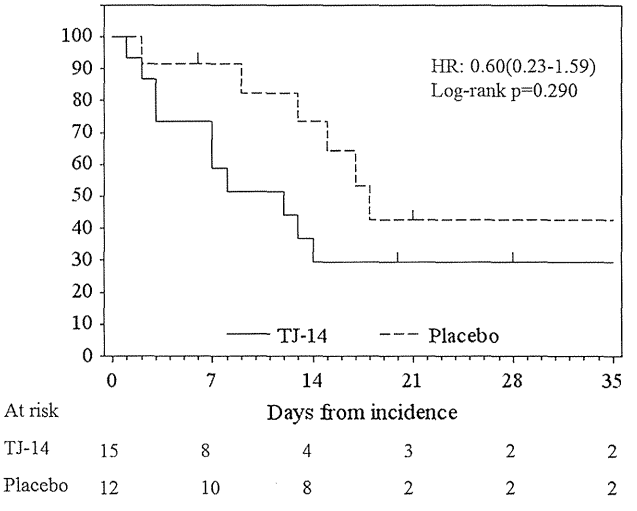

Fig. 2 Duration of any grade of chemotherapy-induced oral mucositis in the patients who developed grade 1 oral mucositis during the screening cycle

treatment-related adverse events were anorexia, a change in PS, nausea, neutropenia, and diarrhea, all of which typically occur in cancer patients receiving cytotoxic chemotherapy (Table 3). The majorities of these events were mild to moderate in severity and considered to be unrelated to the study drug.

Discussion

To date, this randomized trial is the first evaluation of the use of TJ-14 to treat COM in patients with gastric cancer in a prospective placebo-controlled randomized phase II study. The primary purpose of this study was to prove the effects of TJ-14 in reducing the incidence of ≧grade 2 oral mucositis. The incidence of oral mucositis of ≧grade 2 was 40.0 % in the TJ-14 group and 41.3 % in the placebo group in the overall study population. Therefore, treatment with TJ-14 did not exhibit any effect with regard to reducing the frequency of grade 2 events or the duration of grade 2 chemotherapy-induced oral mucositis in gastric cancer patients receiving fluorinated pyrimidine-based chemotherapy. Why did this trial not meet its primary objective? The most likely reason is that the dose reduction of chemotherapy performed before the administration of TJ-14 treatment may have affected the incidence and duration of COM. Generally, among patients who developed ≧grade 2 COM before being entered into this study, the physicians may have been inclined to stop or postpone the

Table 3 Hematological and biochemical toxicities observed during the treatment

	≥Grade 1			≥Grade 2		
	TJ-14 ($N = 45$)	Placebo ($N = 46$)	p value	TJ-14 ($N = 45$)	Placebo ($N = 46$)	p value
Hematological toxicity						
Leucopenia	5 (11 %)	8 (17 %)	0.39	0 (0 %)	1 (2 %)	0.32
Neutropenia	7 (16 %)	7 (15 %)	0.96	3 (7 %)	4 (9 %)	0.72
Hemoglobin	37 (82 %)	40 (87 %)	0.53	13 (29 %)	8 (17 %)	0.19
Platelet	4 (9 %)	6 (13 %)	0.53	0 (0 %)	1 (2 %)	0.32
T-Bilirubin	3 (7 %)	4 (9 %)	0.72	0 (0 %)	0 (0 %)	1.00
AST	2 (4 %)	2 (4 %)	0.98	0 (0 %)	0 (0 %)	1.00
Non-hematological toxicity						
Anorexia	18 (40 %)	19 (41 %)	0.90	8 (18 %)	4 (9 %)	0.20
Nausea	7 (16 %)	9 (20 %)	0.62	2 (4 %)	2 (4 %)	0.98
Vomiting	3 (7 %)	2 (4 %)	0.63	0 (0 %)	1 (2 %)	0.32
Diarrhea	5 (11 %)	4 (9 %)	0.70	0 (0 %)	1 (2 %)	0.32
Constipation	3 (7 %)	5 (11 %)	0.48	0 (0 %)	1 (2 %)	0.32
Peripheral neuropathy	1 (2 %)	1 (2 %)	0.99	0 (0 %)	1 (2 %)	0.32
Lassitude	3 (7 %)	3 (7 %)	0.99	0 (0 %)	1 (2 %)	0.32
Hand-foot syndrome	0 (0 %)	1 (2 %)	0.32	0 (0 %)	1 (2 %)	0.32
Skin reaction	2 (4 %)	0 (0 %)	0.15	0 (0 %)	1 (2 %)	1.00
Dysgeusia	2 (4 %)	1 (2 %)	0.54	1 (2 %)	1 (2 %)	0.99
Edema	1 (2 %)	1 (2 %)	0.99	0 (0 %)	1 (2 %)	0.32
Change in PS	8 (18 %)	9 (20 %)	0.83	2 (4 %)	3 (7 %)	0.66

AST aspartate aminotransferase

original chemotherapy and reduce the dose at the time of the next chemotherapy cycle, which was exactly the time of study treatment and observation [33]. The incidence of oral mucositis of ≧grade 2 was 36.4 % in the patients who received chemotherapy dose reduction and 42.0 % in the patients who did not receive dose reduction. With regard to the incidence of toxicity in this study, 36 patients developed CTCAE v4.0 ≧grade 2 oral mucositis during the screening cycle. Among these patients, seven received dose reduction before the protocol cycle. The median duration of ≧grade 2 oral mucositis was 10.0 days in the patients treated with prophylactic dose reduction and 16.0 days in the patients treated without prophylactic dose reduction. There was a significant difference between the two groups ($p = 0.034$). It has been reported that the COM was depending on the dose and type of chemotherapy [34, 35]. Coleman et al. [36] evaluated 116 women with measurable metastatic breast cancer participated in a randomized phase II study of single-agent liposomal pegylated doxorubicin given either as a 60 mg/m^2 every 6 weeks (ARM A) or 50 mg/m^2 every 4 weeks (ARM B) schedule. They found that the adverse event profiles of the two schedules were distinctly different, and mucositis was more common with ARM A (35 % CTC grade 3/4 in ARM A, 14 % in ARM B). More, Elting et al. [37] retrospectively analyzed 599 patients who developed chemotherapy-induced oral mucositis. They found that a reduction in the dose of the next cycle of chemotherapy was twice as common after cycles with mucositis as it was after cycles without mucositis (23 vs. 11 %; $p \leq 0.0001$). Taking these findings into consideration, dose reduction in the chemotherapy regimen may have been a key issue improving the incidence and/or the duration of COM. We assume that the effects of TJ-14 in oral mucositis may be less prominent due to the use of chemotherapy dose reduction just before the experimental cycles. Taking these findings into consideration, dose reduction in the chemotherapy regimen may have been a key issue improving the incidence and duration of COM. We assume that the effects of TJ-14 in oral mucositis may be less prominent due to the use of chemotherapy dose reduction just before the experimental cycles.

A borderline significant difference, however, was observed in the patients who developed ≧grade 1 COM at the time of screening. The median duration of any grade of oral mucositis was 9.0 days in the TJ-14 group and 17.0 days in the protocol treatment cycle group. Treatment with TJ-14 reduced the duration of any grade of oral mucositis compared with the placebo. In patients with grade 1 COM before the experimental cycle, it is presumed that the physicians may not have reduced the chemotherapy

dose. Therefore, most of the patients who developed COM of grade 1 were not influenced by dose reduction of chemotherapy. These results suggest that the effects of TJ-14 would have been more prominent if chemotherapy dose reduction had not been performed before the experimental cycles. As mentioned above, it has been previously reported that TJ-14 exerts an anti-inflammatory effect by suppressing the levels of lipopolysaccharide-induced IL-6 and IL-8, and cyclooxygenase (COX)-1 and COX-2 [38, 39], in a dose-dependent manner [40]. Further studies are needed to clarify the exact mechanisms underlying these observations.

In conclusion, this trial did not show a beneficial effect of TJ-14 in reducing the incidence of chemotherapy-induced oral mucositis as the primary end point, likely due to the use of dose reduction before the experimental cycles, which was not prohibited by the study protocol. In the patients with >grade 1 COM at the screening cycle, an obvious reduction in the risk of COM (HR 0.60) was demonstrated. In this regard, the addition of TJ-14 to chemotherapy regimens may have shortened the duration of oral mucositis when no dose reduction was performed before the administration of the experimental agents. A further analysis may lead to a better interpretation of the study results by examining subgroups that will benefit from TJ-14 treatment. A more definitive design in a future trial of TJ-14 for chemotherapy-induced oral mucositis is needed to eliminate the influence of arbitrary dose reduction based on the discretion of the individual physician.

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Conflict of interest None declared.

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