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ORIGINAL ARTICLE

A multicenter phase II study of combined chemotherapy with docetaxel, cisplatin, and S-1 in patients with unresectable or recurrent gastric cancer (KDOG 0601)

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Abstract
Purpose We conducted a phase II study to evaluate the efficacy and safety of a triplet regimen of docetaxel, cisplatin, and S-1 in patients with unresectable or recurrent gastric cancer.
Methods Docetaxel (40 mg/m^2) and cisplatin (70 or 60 mg/m^2) were given on day 1 of a 28-day cycle. S-1 (40 mg/m^2) was given twice daily on days 1–14. Treatment with this regimen was continued for a maximum of 6 cycles. Subsequently, patients with no disease progression received a combination of docetaxel and S-1.
Results Fifty-nine patients were enrolled. The median number of administered cycles was 8 (range, 1–25). Because some patients had serious myelosuppression and renal dysfunction with 70 mg/m^2 of cisplatin, dose of cisplatin was reduced to 60 mg/m^2 after 19 patients had been treated. Common severe toxic effects of grade 3 or 4 were leukocytopenia (44%), neutropenia (72%), anemia (15%), and febrile neutropenia (14%). The overall response rate of this group was 81% (95% confidence interval (CI), 71–91%). The median overall survival and progression-free survival were 18.5 (95% CI, 15.6–21.5) and 8.7 (95% CI, 6.7–10.7) months, respectively.
Conclusions Triplet of docetaxel, cisplatin, and S-1 is a well-tolerated and highly active regimen for advanced or recurrent gastric cancer. A 60 mg/m^2 of cisplatin is as effective as 70 mg/m^2 of cisplatin.

Keywords Docetaxel · Cisplatin · S-1 (combination) · Gastric cancer · Phase II

Introduction

Gastric cancer, the most common malignant tumor arising in the gastrointestinal tract, is the second leading cause of cancer-related death in the world, after lung cancer. There are about 700,000 deaths from gastric cancer per year [1, 2]. The 2009 edition of "Vital statistics of Japan" published by the Ministry of Health, Labour and Welfare estimated that in 2007, there were 50,597 deaths from gastric cancer in Japan, accounting for 15% of all cancer-related deaths [3]. Similar to international trends, mortality from gastric cancer is second highest, following that from lung cancer in Japan. A further decrease in mortality would require improved treatment outcomes in patients with unresectable advanced or recurrent gastric cancer.

S-1 is an oral fluoropyrimidine derivative developed in Japan, based on the concept of biochemical modulation. S-1 consists of the following three components in a molar ratio of 1:0.4:1: tegafur, a prodrug which slowly

This study has been registered with UMIN Clinical Trials Registry (UMIN-CTR), number UMIN000001119.

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metabolized to 5-fluorouracil; gimeracil, which reversibly inhibits dihydropyrimidine dehydrogenase, the rate-limiting degrading enzyme of 5-fluorouracil, thereby increasing the plasma concentration of 5-fluorouracil; and oteracil potassium, which is distributed in high concentrations in gastrointestinal tissue and inhibits phosphorylation of 5-fluorouracil, thereby reducing gastrointestinal toxicity. It was developed to achieve enhanced efficacy with less toxicity when compared to conventional 5-fluorouracil derivatives [4].

In 2007, the Japan Clinical Oncology Group (JCOG) 9912 study reported that the therapeutic efficacy of S-1 monotherapy was noninferior to 5-fluorouracil alone regimen, with a better toxicity profile. The study concluded that S-1 should be a new standard treatment option for advanced gastric cancer [5].

In addition, we also performed a phase III study comparing S-1 plus cisplatin with S-1 alone in patients with advanced gastric cancer (SPIRITS trial). The study demonstrated significantly improved survival with S-1 plus cisplatin compared to S-1 alone [6]. At present, S-1 plus cisplatin is recognized as a standard treatment for unresectable, advanced, or recurrent gastric cancer in Japan. In 2009, the results of the First-Line Advanced Gastric Cancer Study (FLAGS) comparing 5-fluorouracil plus cisplatin with S-1 plus cisplatin were reported. S-1 plus cisplatin was shown to be at least equivalent to 5-fluorouracil plus cisplatin [7]. Because of its good toxicity profile, S-1 plus cisplatin is expected to be used as a first-line treatment in countries other than Japan, especially in East Asia in the near future. However, the efficacy of S-1 plus cisplatin is still unsatisfactory. Development of new treatment regimens is essential for a further decrease in mortality from gastric cancer.

A triplet regimen of 5-fluorouracil, cisplatin, and docetaxel (DCF) is one of the standard treatments for unresectable advanced gastric cancer in Western countries. DCF was associated with significantly better outcomes when compared to 5-fluorouracil plus cisplatin, indicating that the addition of docetaxel in the triplet regimen enhanced effectiveness [8]. We have therefore started to study the effect of adding docetaxel to base treatment with S-1 plus cisplatin to further improve outcomes. Since DCF was reported high hematotoxicity, we adopted 4-weekly regimen, which has 14 days of rest, to manage toxicity and reduce treatment delay, not 3-weekly regimen. And docetaxel and cisplatin was administered on day 1 in terms of convenience. We previously performed a phase I study to evaluate the safety and to determine the maximum tolerated dose and recommended dose of triplet regimen with docetaxel, cisplatin, and S-1 (DCS). DCS was highly active with acceptable toxicity in that phase I study [9]. On the basis of these results, we performed this multicenter single-arm phase II study.

Patients and methods

Patients

Patients had to meet the following eligibility criteria: (1) unresectable or recurrent gastric cancer with a histopathologically confirmed diagnosis of adenocarcinoma; (2) the presence of measurable lesions within 28 days before enrollment; (3) no previous therapy (radiotherapy, chemotherapy, or hormone therapy) for the gastric carcinoma; (4) age between 20 and 80; (5) no severe vital organ dysfunction (bone marrow, heart, lungs, liver, kidneys, etc.), i.e., a leukocyte count $\geq 3 \times 10^3/\mu L$, a platelet count $\geq 100 \times 10^3/\mu L$, a serum total bilirubin concentration ≤ 1.5 mg/dL, serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) concentration ≤ 100 IU/L (in patients with liver metastasis, however, AST and ALT concentration of not more than five times of the upper limit of normal at the institution performing the test), a serum creatinine concentration ≤ 1.5 mg/dL, a serum creatinine clearance (24 h urine specimen) ≥ 50 mL/min, and a normal electrocardiogram; (6) a performance status (Eastern Cooperative Oncology Group scale) of 0–2; (7) being able to tolerate oral intake; (8) life expectancy of at least 8 weeks from the date of enrollment; and (9) written informed consent from each patient.

Ethical, medical, and scientific aspects of the study were reviewed and approved by the ethics committees of each participating institution. The study was conducted in accordance with the declaration of Helsinki of 1975, revised in 2000.

Treatment schedule

DCS was administered as per the doses determined in our previous phase I study. S-1 (body surface area [BSA] <1.25 m^2, 40 mg; BSA ≥ 1.25 to <1.5 m^2, 50 mg; and BSA ≥ 1.5 m^2, 60 mg) was given orally twice daily after breakfast and dinner for 14 consecutive days, followed by 14 days of rest. Docetaxel (40 mg/m^2) was given as a continuous intravenous infusion over the course of at least 60 min on day 1. Cisplatin (70 or 60 mg/m^2) was given as a continuous intravenous infusion over the course of at least 90 min on day 1 with adequate hydration. Treatment with triplet therapy was continued for a maximum of 6 cycles. Subsequently, patients received a combination of docetaxel and S-1 until disease progression.

The doses of both S-1 and cisplatin were reduced in patients who had any of the following: a leukocyte count of less than $1.0 \times 10^3/\mu L$, a neutrophil count of less than $500/\mu L$, a platelet count of less than $2.5 \times 10^4/\mu L$, grade 3 or 4 febrile neutropenia, or grade 3 or 4 nonhematological toxicity except for nausea, vomiting, and anorexia, or if the

start or resumption of treatment had to be delayed for at least 8 days because of toxicity. The dose of S-1 was decreased in a stepwise fashion by up to 2 levels as follows: BSA <1.25 m^2, from 40 to 25 and 20 mg/dose; BSA ≥1.25 to <1.5 m^2, from 50 to 40 and 25 mg/dose; and BSA ≥1.5 m^2, from 60 to 50 and 40 mg/dose. In addition, the dose of cisplatin was decreased in a stepwise fashion by 10 mg/m^2 each, and treatment was continued. In patients who had a serum creatinine level of ≥2 mg/dL or grade 4 anorexia caused by cisplatin, only the dose of cisplatin was reduced. Treatment was discontinued in case of any of the following conditions: distinct evidence of disease progression; development of complications, treatment-related death, or septic shock; the patient refused to continue the study treatment or withdrew consent; postponement of the resumption of treatment for 2 or more weeks.

As supportive treatment for grade 4 neutropenia and grade 3 or 4 febrile neutropenia, granulocyte colony-stimulating factor (G-CSF) and antibiotics administration was used at the investigator's discretion. Prophylactic G-CSF was not allowed.

Toxicity assessment

Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (version 3.0). During protocol treatment, signs and symptoms, blood counts, liver function, renal function, and electrolytes were assessed once a week.

Response evaluation

Tumor responses to chemotherapy were evaluated according to the guideline of the Response Evaluation Criteria in Solid Tumors (RECIST). Responses were evaluated by computed tomography every 2 months. First, we evaluated in the 1st and 2nd courses, afterward every 2 courses. Radiographs of all evaluable patients were reviewed externally to confirm investigator-designated responses by the independent review committee. Downstaging was defined as the case deemed to be disappeared unresectable factor and to be resectable by computed tomography and magnetic resonance imaging. Progression-free survival was defined as the time from start of treatment to tumor progression or death for any causes that occurs by the end of the study. Patients with no confirmation of progression or death were censored at the date of the last objective tumor assessment. Overall survival was defined as the time from start of treatment to the date of death. If the death has not occurred, the survival time was censored on the last date the patient has known to be alive.

Statistical analysis

The primary endpoint of this study was the objective response rate. Secondary endpoints were safety, progression-free survival, and overall survival. Because the response rates with S-1 plus docetaxel were 46 and 56.3% in previously reported phase II studies [10, 11], we hypothesized that it would be worthwhile to pursue a phase III study if the response rate reached 55% in the present study. We therefore assumed an expected response rate of 55% and a threshold response rate of 30%, with 1-sided alpha error of 0.05 and a beta error of 0.1. The required number of patients was estimated to be 35. Forty patients were required with the inclusion of about 10% follow-up loss. An interim analysis was scheduled to be performed after the enrollment of 20 patients. If the number of patients with a complete or partial response was five or less, the protocol specified that the study was to be discontinued.

Results

Patient characteristics

From October 2006 through August 2008, 59 patients (47 men and 12 women) were enrolled in the study. Table 1 shows the demographic characteristics of the patients. The performance status was 0 in 40 patients, 1 in 18, and 2 in 1. The histological types were intestinal in 25 patients and diffuse in 34. The median number of successive treatment cycles per patient was 8 (6 for DCS therapy and 2 for docetaxel plus S-1; range, 1–25). An interim analysis was performed after 19 patients had been enrolled and confirmed that 15 patients had a partial response. When 19 patients were enrolled, 5 had grade 4 febrile neutropenia. Because it had been judged that examination by the data and safety monitoring board was necessary, an interim analysis was conducted in 19 patients. The criteria for early discontinuation of the study as specified by the protocol were thus not met, and enrollment was continued.

Treatment result

The total treatment cycle of DCS was 514, and the median treatment cycle was 8 (1–25). Dose reductions were required in 25 patients (42%), and relative dose intensities of S-1, docetaxel, and cisplatin were 94.8, 99.0, and 89.9%, respectively. Treatment had to be delayed by 8 or more days in 3 patients. There was one case of treatment discontinuation and drug-related death caused by the perforation

Table 1 Patient characteristics

Patients	$n = 59$
Age (range)	62 (35–75)
Gender M/F	47/12
PS 0/1/2	40/18/1
Metastatic/recurrence	49/10
Histological type	
Intestinal type	25
Diffuse type	34
Metastatic site	
Liver	33
Lymph node	46
Ovary	3
Lung	2
Peritoneum	17
Other	2
CDDP dose (mg)	
60/70	40/19

of the primary tumor. However, this patient refused surgery.

Adverse events

In this phase II study, the initially used dose of cisplatin was 70 mg/m^2, the recommended dose determined in our previous phase I study [9]. After 19 patients had been enrolled, 15 (79%) had grade 3 or higher neutropenia, and 5 (26%) had grade 1 renal dysfunction (elevated creatinine clearance). The dose of cisplatin was therefore reduced to 60 mg/m^2, and the study was continued. And again, the study was continued until the target number.

In the study group as a whole, the incidences of grade 3 or higher adverse events were as follows: leukocytopenia, 44%; neutropenia, 73%; anemia, 15%; febrile neutropenia, 14%; anorexia, 7%; nausea, 5%; vomiting, 3%; fatigue, 2%; and diarrhea, 5%. In patients given 60 mg/m^2 of cisplatin, the incidences of all toxic events were lower than those in patients given 70 mg/m^2 of cisplatin (Table 2). G-CSF and antibiotics were administered to patients who had grade 4 neutropenia and grade 3 or 4 febrile neutropenia ($n = 21$; 12 for CDDP 60 mg/m^2, and 9 for CDDP 70 mg/m^2).

Efficacy

In the study group as a whole, the response rate according to the dose of cisplatin was 79% (95% confidence interval, 61–97%) for 70 mg/m^2 and 83% (95% confidence interval, 71–94%) for 60 mg/m^2. Use of the lower dose of cisplatin thus did not negatively affect the response (Table 3). We could not evaluate one patient because of treatment-related death.

The median overall survival and median progression-free survival were 18.5 months (95% confidence interval (CI), 15.6–21.5) and 8.7 months (95% CI, 6.7–10.7), respectively, during a median follow-up period of 18.5 (95% CI, 0.4–42.3) months (Fig. 1a, b).

Table 2 Adverse events

	CDDP: 70 mg ($n = 19$)					CDDP: 60 mg ($n = 40$)					Overall ($n = 59$)				
	G1	G2	G3	G4	≥G3 (%)	G1	G2	G3	G4	≥G3 (%)	G1	G2	G3	G4	≥G3 (%)
Hematological toxicity															
Leukopenia	1	4	10	2	12 (63)	6	15	13	1	14 (35)	7	19	23	3	26 (44)
Neutropenia		2	7	8	15 (79)	4	7	15	13	28 (70)	4	9	22	21	43 (73)
Anemia	5	7	6		6 (32)	4	18	3		3 (8)	25	15	9		9 (15)
Thrombocytopenia	10	5				17	3				27	8			
Febrile neutropenia				5	5 (26)			3		3 (8)			8		8 (14)
Nonhematological toxicity															
AST/ALT	5	3	2		2 (11)	12	1		1	1 (3)	17	4	2	1	3 (5)
Cr	4	1				6					10	1			
Stomatitis	2					7	3				9	3			
Anorexia	10	5	2		2 (11)	26	10	2		2 (5)	36	15	4		4 (7)
Nausea	8	5	1		1 (5)	26	5	2		2 (5)	34	10	3		3 (5)
Vomiting	6	2	1		1 (5)	12	2	1		1 (3)	18	4	2		2 (3)
Fatigue	7	1				14	3	1		1 (3)	21	4	1		1 (2)
Diarrhea	4	1	1		1 (5)	4	5	2		2 (5)	8	6	3		3 (5)

n number of patients, *G1–G4* grades 1–4, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Table 3 Response rate

	n	CR	PR	SD	PD	NE	RR (%)
Overall	59	0	48	10	0	1	81
CDDP 60 mg	40	0	33	6	0	1	83
CDDP 70 mg	19	0	15	4	0	0	79
Liver	31	1	26	3	0	1	87
Lymph node	45	1	35	8	0	1	80
Others	6	1	2	3	0	0	50

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *RR* response rate

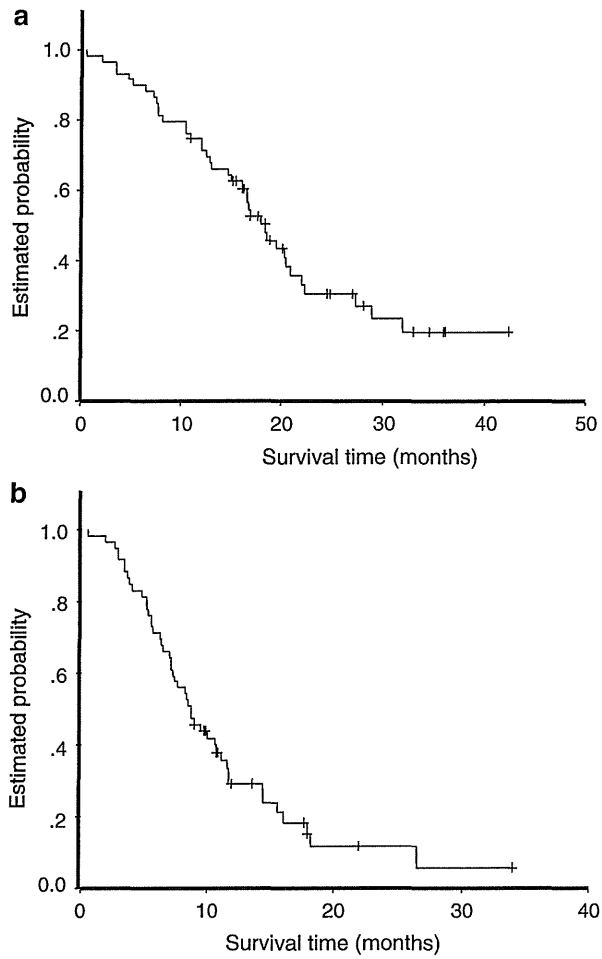

Fig. 1 Kaplan–Meier curves for **a** overall survival and **b** progression-free survival

Second-line treatment

Of the 46 patients who had disease progression during the study, 39 (85%) could receive second-line treatment. Thirty-four of these patients received irinotecan-based regimens (irinotecan alone in 19; irinotecan and cisplatin in 12; irinotecan, 5-fluorouracil, and *l*-leucovorin in 2; and irinotecan and mitomycin C in 1), 4 received S-1 (adjuvant chemotherapy after surgery in 2; modification because of toxicity in 2), and 1 received methotrexate plus 5-fluorouracil.

Discussion

We conducted this phase II study to investigate the efficacy and safety of triplet regimen with DCS in patients with unresectable advanced or recurrent gastric cancer. The response rate was 81%, and the disease control rate was 98%. The median overall survival and progression-free survival were 18.5 and 8.7 months, respectively. Our regimen was effective and feasible as a first-line treatment of advanced or recurrent gastric cancer.

In 2007, the SPIRITS trial demonstrated the superiority of S-1 plus cisplatin regimen as compared with S-1 alone, with a response rate of 53% and a median survival time of 13 months [6]. In Japan, S-1 plus cisplatin is recognized as a standard treatment. On the other hand, doublet regimens combining S-1 with drugs other than cisplatin have been studied extensively. With a combination of S-1 and docetaxel, Yoshida et al. [10] obtained a response rate of 56.3% with overall survival of 14.3 months, and Yamaguchi et al. [11] reported a response rate of 46% with overall survival of 14 months in phase II studies. These results were similar to those obtained with S-1 plus cisplatin. Because of its high antitumor activity and good tolerance, S-1 plus docetaxel is expected to be used as first-line treatment. At present, the START trial, a multicenter, collaborative, phase III study designed to validate the superiority of a combination of S-1 plus docetaxel over S-1 alone (used as a control) in terms of therapeutic usefulness, is ongoing in a Japan–Korea collaborated trial [12].

Van Cutsem et al. [8] conducted a phase III controlled study (V 325) to compare 5-fluorouracil plus cisplatin with DCF therapy as first-line treatment in patients with unresectable advanced gastric cancer. DCF therapy was associated with significantly better outcomes than 5-fluorouracil plus cisplatin, demonstrating that triplet therapy was more effective. As mentioned above, S-1 is a widely used as a key drug for the treatment of gastric cancer in Japan. Since TS-1 was blended gimeracil which was DPD inhibitor, in diffuse type which DPD had high expression, TS-1 was shown higher effectiveness when compared to 5-FU. Moreover, in JCOG9912, the tendency with S-1 better than 5-FU is looked in OS by the track result. Also, in the FLAGS carried out by global study, S-1 was shown better result in diffuse type when compared to 5-FU. So, we believed that antitumor effectiveness would be enhanced by substituting S-1 for 5-fluorouracil in DCF and therefore planned phase I and II clinical trials of triplet therapy with DCS.

Table 4 Adverse events (first 2 courses)

	CDDP: 70 mg (n = 19)					CDDP: 60 mg (n = 40)					Overall (n = 59)				
	G1	G2	G3	G4	>G3 (%)	G1	G2	G3	G4	>G3 (%)	G1	G2	G3	G4	>G3 (%)
Hematological toxicity															
Leukopenia	2	4	7	1	8 (42)	9	11	4	1	5 (13)	11	15	11	2	13 (22)
Neutropenia		5	7	3	10 (53)	8	8	11	3	14 (35)	8	13	18	6	24 (41)
Anemia	3	9	2		2 (11)	10	3	1		1 (3)	13	12	3		3 (5)
Thrombocytopenia	9	1				7	2				16	3			
Febrile neutropenia			4		4 (21)								4		4 (7)
Nonhematological toxicity															
AST/ALT	3	2	2		2 (11)	8			1	1 (3)	11	2	2	1	3 (5)
Cr	3					4					7				
Stomatitis	1					6	2				7	2			
Anorexia	7	5	2		2 (11)	26	7	1		1 (3)	33	12	3		3 (5)
Nausea	6	5	1		1 (5)	19	5	2		2 (5)	25	10	3		3 (5)
Vomiting	4	1	1		1 (5)	8	2	1		1 (3)	12	3	2		2 (3)
Fatigue	6	1				13	3				19	4			
Diarrhea	2	1	1		1 (5)	2	2	2		2 (5)	4	3	3		3 (5)

n number of patients, *G1–G4* grades 1–4, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

In a phase I study designed to evaluate the optimal dose and dose-limiting toxicity of DCS therapy, the recommended dose of cisplatin was determined to be 70 mg/m^2 [9]. This dose was used in the present phase II study. During the study, an interim analysis was performed according to the protocol to assess the safety and efficacy of DCS therapy. Grade 1 or higher renal dysfunction occurred in 26% of the patients, and grade 3 or higher neutropenia occurred in 79%. The dose of cisplatin was therefore reduced to 60 mg/m^2. This lower dose of cisplatin was associated with a trend toward less toxicity, with no change in the response rate. We therefore consider 60 mg/m^2 of cisplatin to be a reasonable dose for future studies. Although caution is required when comparing the results of different studies, DCS regimen in the present study expected to be more effective than S-1 plus cisplatin in the SPIRITS study.

There are also limitations when comparing our results with those of a previous phase III study, but the V325 study reported that DCF had a response rate of 38.7%, a median progression-free survival of 5.2 months, and a median survival time of 10.2 months. As compared with these results, DCS was promising regimen. DCF was also associated with many serious adverse events, such as neutropenia (82%) and leukopenia (65%), indicating some problems in tolerability. With our DCS regimen, main serious adverse events were also neutropenia (73%) and febrile neutropenia (14%). These toxicities did not lead to discontinuation of treatment due to G-CSF administration and dose reduction of CDDP and S-1. Now phase II study of 2 courses of DCS as neoadjuvant setting for operable gastric cancer with extensive lymph node metastasis is planned by JCOG. Focusing on the first 2 courses with toxicity, DCS was more feasible (Table 4).

A phase II study of triplet regimen of docetaxel, CDDP, and S-1 has also been performed by Sato et al. S-1 was administered orally twice daily on days 1–14 at a dose calculated according to the patient's body surface area as follows: <1.25 m^2, 40 mg; 1.25–1.5 m^2, 50 mg; and >1.5 m^2, 60 mg. CDDP was administered, followed by docetaxel at 60 mg/m^2 on day 8. Cycles were repeated every 3 weeks. They reported a response rate of 87.1% and a disease control rate of 100%. The median overall survival and progression-free survival were 687 days and 226 days, respectively [13]. Although the treatment regimen differed from ours, their DCS regimen was also shown to be effective, consistent with the results of our study. We believe that the high effectiveness of these triplet regimens is reproducible. Both DCS regimens indicated not only high response rate and long PFS but also long OS over 18 months. However, this longer OS is interpreted with caution. According to the NCDB data, prognosis in early stage in Asian race is longer than in other races, but that of Stage IV is similar in Asian and other races [14]. Otherwise, in several trials, overall survival in Japanese trials is longer than those in multinational trials. We speculate that high percentage of patients received second line in Japan might contribute to prolonged survival. And in this study, it may be related to the cases had taken surgery because of the high response rate.

In conclusion, DCS is a regimen that is expected to be highly effective with manageable toxicities. To confirm the therapeutic usefulness of DCS for the first-line treatment of advanced or recurrent gastric cancer, we are also now planning a multicenter, phase III clinical trial comparing with cisplatin plus S-1 as reference arm, currently a standard treatment in Japan.

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Conflict of interest None.

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Mini-review

Human gastric cancer development with TNF-α-inducing protein secreted from *Helicobacter pylori*

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ABSTRACT

TNF-α-inducing protein (Tipα) is a unique carcinogenic factor of *Helicobacter pylori*, which is secreted into culture broth. The biological activities of Tipα and deletion mutant were studied. Tipα protein specifically binds to cell-surface nucleolin and then enters the gastric cancer cells, where *TNF-α* and *chemokine* gene expressions are induced by NF-κB activation. Nucleolin localizes on the surface of gastric cancer cells, and interaction between Tipα and cell-surface nucleolin causes a cancer-oriented microenvironment that increases the risk of gastric cancer. This paper discusses a new mechanism of gastric cancer development with *H. pylori* and provides a new preventive strategy.

1. Introduction

Helicobacter pylori (*H. pylori*) is the gram-negative micro-aerophilic bacterium that attaches to gastric epithelial cells in the human stomach. It is the only bacterium to be classified as a definitive carcinogen (group 1) for humans by the World Health Organization's International Agency for Research on Cancer. Approximately 75% of all gastric cancer cases in the world are directly attributed to *H. pylori* infection [1–4], and it is well understood that persistent *H. pylori* infection induces chronic inflammation in the stomach and duodenum, associated with strong induction of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), and also chemokines [3–6]. Experiments with Mongolian gerbils have indicated that *H. pylori* infection induces tumor promotion in stomach initiated with various carcinogens [7,8]. However, the exact mechanism of how inflammation by *H. pylori* infection promotes human cancer development is not known and is therefore an urgent research objective. Our experiments with TNF-α-deficient mice have revealed that TNF-α is one of the essential cytokines for tumor promotion, and that a cytokine network sequence of tumor promotion begins with TNF-α and runs through IL-1 and IL-6 [9,10]. *H. pylori* gene product associated with TNF-α-inducing activity may play a key role in tumor promotion of gastric cancer. Therefore, we cloned a new gene (*Hp0596*) from genome sequence of *H. pylori* strain 26695 and named it the *TNF-α-inducing protein* (*Tipα*) gene [11]. Tipα protein of the recombinant gene strongly induces both *TNF-α* gene expression by NF-κB activation in mouse gastric cancer cells MGT-40, and transformation of Bhas 42 cells (*v-H-ras* transfected BALB/3T3 cells) [11]. Thus Tipα is considered to be a carcinogenic factor of *H. pylori* associated with tumor promoting activity.

Tipα gene is unique to the *H. pylori* genome, and Tipα protein has no similarity to any other virulence factors of *H. pylori*, such as immunodominant cytotoxin-associated antigen (CagA), vacuolating cytotoxin (VacA), or urease. Tipα protein is secreted from *H. pylori* in culture broth, not mediated through Type IV secretion system. It induces activation of NF-κB in a cag pathogenicity island (cagPAI) independent manner [11–13]. Recently we identified nucleolin on the cell surface as a receptor of Tipα. Nucleolin shuttles Tipα protein from membrane to cytosol, and then to nuclei, inducing the activation of NF-κB [14]. Nucleolin is a well-known major non-ribosomal protein in nucleoli, and it has multi-functions, including chromatin remodeling, DNA recombination, rRNA processing, and mRNA stabilization [15,16]. Numerous investigations have reported that nucleolin is localized on the membrane surface of various cancer cells, where it acts as a receptor for various molecules [17–21]. Nucleolin is present on the surface of human gastric cancer cell lines [22].

In this Mini-review, we report on unique features of Tipα: (1) Proteins of *Tipα* gene family and the crystal structure, (2) Mechanism of *TNF-α* and *chemokine* gene expressions mediated through

Abbreviations: TNF-α, Tumor necrosis factor-α; Tipα, TNF-α inducing protein.

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NF-κB activation, (3) Transforming activity and relevance to human gastric cancer development and (4) Cell surface nucleolin as a specific receptor of Tipα. The significance of Tipα and nucleolin as molecular targets for prevention and treatment of gastric cancer is also discussed.

2. Proteins of *Tipα* gene family and the crystal structure

H. pylori contains proteins of *Tipα* gene family, such as *Tipα* itself, isolated from strain 26695, *H. pylori-membrane protein* (*HP-MP1*), from strain SR7791, and *jpg0543*, from strain J99 [11,13,23]. 94.8% of their protein sequences are identical (Fig. 1A). All examined *H. pylori* strains contain *Tipα* gene, but recent phylogenetic analysis using 20 complete genome sequences of *H. pylori* strains revealed that *Tipα* gene is one of the genes that greatly diverged from East Asian strains and European strains, in addition to *cagA* and *vacA* [24]. It is important to understand that the knockout of *Tipα* gene decreases the colonizing activity of *H. pylori* in mouse stomach [25]: Tipα plays a carcinogenic role in *H. pylori*-infection.

Tipα protein, with a molecular weight of 19 kDa, is secreted from *H. pylori* as a 38 kDa homodimer, which is active in the induction of *TNF-α* gene expression in mouse gastric cancer cell line MGT-40 cells. A deletion mutant of Tipα (del-Tipα), with six amino acids deleted including two cysteine residues from *N*-terminal, and Cys5Ala/Cys7Ala double mutant (C5A/C7A-Tipα) induced weaker activity than Tipα in both *TNF-α* gene expression and transforming activity. Further studies with mutants of Tipα have indicated that homodimer formation by one disulfide bond is required to maintain the activity [11,12].

We report the crystal structure of del-Tipα because Tipα did not crystallize well due to its tendency to aggregate. X-ray crystallographic analysis revealed that del-Tipα has a unique elongated structure containing a 40-Å long α helix (α1) and β1-α1-α2-β2-β3-α3-α4 topology with the *N*-terminal flexible region (Fig. 1B) [26]. del-Tipα forms a heart shaped homodimer without covalent bonds, and other X-ray crystallographic analyses with truncated forms of Tipα have also reported that it takes dimerized forms without any cysteine residues [27,28]. Recent bioinformatics have indicated that an intrinsically disordered segment of protein is significant for biological activity [29]. The *N*-terminal flexible region of del-Tipα, as shown in Fig. 1B, is also assumed to be important for the activity. Whether the crystal structure of Tipα is similar to that of del-Tipα, remains to be investigated.

3. Mechanism of *TNF-α* and *chemokine* gene expressions mediated through NF-κB activation

The activity of Tipα has been confirmed by two different experiments. Firstly, the recombinant Tipα protein at concentrations of 50 μg/ml (2.6 μM) and 100 μg/ml (5.2 μM) induced about 18.9- and 25.7-fold increase *TNF-α* expression in MGT-40 cells, respectively, after 1 h treatment (Table 1), whereas del-Tipα induced weak activity (Table 1) [11]. Secondly, *TNF-α* gene was more strongly expressed in Bhas/mp1 clones, which are Bhas 42 cells transfected with *HP-MP1* gene (one of *Tipα* gene family), than in Bhas/vec clones (Bhas 42 cells transfected with vector alone) and Bhas/ure clones (Bhas 42 cells transfected by *urease* gene) [23]. Transfection of *Tipα* gene into BALB/3T3 cells (containing no *v-H-ras* gene) did not show any significant induction of *TNF-α* gene

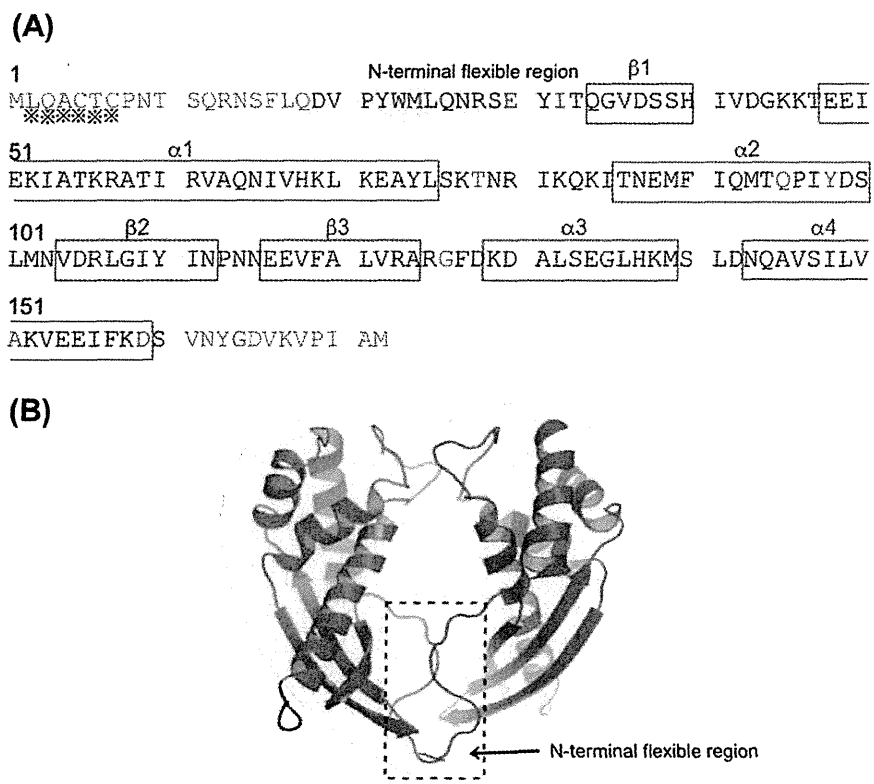

Fig. 1. Primary structure of Tipα (A) and tertiary structure of Tipα (B). (A) Red characters in the sequence indicate different amino acids among proteins of *Tipα* gene family (Tipα, HP-MP1 and jhp0543). The secondary structures are shown in red (α-helix) and blue (β-sheet), and the *N*-terminal flexible region is shown in green. The *N*-terminal and *C*-terminal regions not visible on the electron density map are shown in gray. Six amino acids, indicated as ※ are deleted in del-Tipα. (B) Tertiary structure of del-Tipα dimer was determined by crystal structural analysis.

Table 1
Induction of *TNF-α* and *chemokine* gene expressions and NF-κB activation.

	Tipα	del-Tipα
Cytokine gene (fold)		
TNF-α	25.7	7.0
IL-6	4.6	1.5
Chemokine gene (fold)		
ccl2	28.0	4.0
ccl7	12.0	2.2
ccl20	52.0	4.0
cxcl1	162.0	17.0
cxcl2	314.0	20.0
cxcl5	48.0	6.4
cxcl10	17.0	3.2
NF-κB activation (fold)	2.0	1.1

expression, suggesting that the cooperation of Ras protein is necessary for *TNF-α* gene expression in the cells [23].

Comprehensive gene expression analysis using DNA microarray revealed that Tipα induces *chemokine* gene expressions, such as *ccl2, ccl7, ccl20, cxcl1, cxcl2, cxcl5* and *cxcl10* in MGT-40 cells (Table 1) [30]. Moreover, MGT-40 cells treated with Tipα showed up-regulation of 120 gene expressions (over 2-fold), with 18 genes among them expressed more than 5-fold, including five *chemokine* genes. The expression level of the genes was quantitatively confirmed by real-time PCR, as shown in Table 1 [30]. del-Tipα induced two *cytokine* and *chemokine* gene expressions much more weakly than Tipα did. The results suggest that induction of *chemokine* gene expressions in gastric epithelial cells by *H. pylori* infection is directly involved in the development of gastric cancer, since chemokines (chemoattractant cytokines) are associated with potent leukocyte activation [31,32]. Our results indicate that the expression of *chemokine* genes induced by Tipα also plays a key role in the inflammation and cancer development induced by *H. pylori* infection.

KeyMolnet analysis is a new approach to mechanistic analysis of DNA microarray data developed by the Institute of Medicinal Molecular Design, Inc. (IMMD). KeyMolnet analysis strongly suggests that NF-κB plays a central role in Tipα-induced gene expressions [30]. MGT-40 cells treated with recombinant Tipα induced degradation of IκB and translocation of NF-κB p65 subunit into nuclei, and Tipα treatment induced 2-fold NF-κB activation, determined by NF-κB binding to the consensus sequence (Table 1) [12]. Pre-treatment with the proteasome inhibitor MG-132 significantly inhibited IκB degradation and NF-κB activation, resulting in reduction of gene expressions. Furthermore, Surface Plasmon Resonance assay revealed that Tipα directly binds to $(dGdC)_{10}$ 2400 times more strongly than del-Tipα does, showing the DNA-binding activity of Tipα [33]. Whether Tipα directly activates NF-κB by binding to the consensus sequence needs further investigation.

4. Transforming activity and relevance to human gastric cancer development

The transforming activity of proteins, such as hepatitis C virus core protein and the leukemia-related protein MGT8 (ETO), are demonstrated in Bhas 42 cells *in vitro*, which are BALB/3T3 cells transfected with *v-H-ras* gene [13]. We first reported the malignant transformation of Bhas/mp1 clones that were transfected by *HP-MP1* gene into Bhas 42 cells in nude mice by subcutaneous implantation, and also the anchorage-independent growth of Bhas/mp1 clones in soft agar. The transformed cells strongly induced *TNF-α* gene expression [23]. Furthermore, treatment of Bhas 42 cells with recombinant Tipα protein significantly increased the number of transformed foci, whereas that with recombinant del-Tipα did not [11]. The number of transformed foci induced with Tipα at a 2.6 μM is about half that induced with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a classical tumor promoter, at 1.6 μM (Table 2), suggesting that Tipα has potent tumor promoting activity.

To understand the significance of Tipα in human gastric cancer development, the difference in production and secretion of Tipα into culture broth was determined among *H. pylori* clinical isolates that were established from biopsy samples of gastric mucosa of patients with chronic gastritis or gastric cancer. All *H. pylori* isolated from 17 gastric cancer patients and 11 chronic gastritis patients produced Tipα protein, although the amounts of Tipα varied in culture broth. Tipα secreted from *H. pylori* obtained from gastric cancer patients were higher than those from gastritis patients [12]. Moreover, three *H. pylori* isolates from 11 gastritis patients who later developed gastric cancer also showed larger amounts of Tipα, similar to those from gastric cancer patients (Fig. 2). These results strongly indicate that Tipα secreted from *H. pylori* is a new carcinogenic factor in human gastric cancer development.

5. Cell surface nucleolin as a specific receptor of Tipα

Fluorescence-labeled Tipα specifically binds to the surface of MGT-40 cells and enters the cytosol and nuclei, whereas both del-Tipα and C5A/C7A-Tipα bind weakly to the cells, indicating that a specific binding molecule is present on the cell surface involved in biological activity of Tipα [12]. Nucleolin has been identified as a specific receptor of Tipα, using pull-down assay with anti-FLAG antibody against FLAG-tagged Tipα protein (Tipα-FLAG) [14]. After treatments of human gastric cancer cell line MKN-1 and human monocytic leukemia cell line THP-1 with Tipα-FLAG,

Table 2
Transforming activity of Tipα compared with TPA, a classical tumor promoter, in Bhas 42 cells.

	Concentration		Transforming activity
	μg/ml	μM	(no. of foci)
Tipα	50	2.6	18.0
TPA	1	1.6	38.0

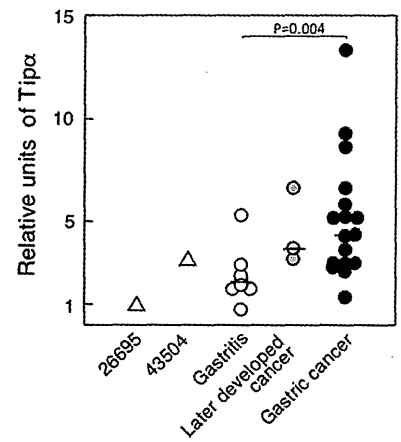

Fig. 2. Secretion of large amounts of Tipα from *H. pylori* clinical isolates obtained from gastric cancer patients. Relative units of Tipα in culture broth were measured by Western blotting, and that in culture broth of strain 26695 was expressed as 1. Each dot corresponds to Tipα in culture broth from each clinical isolate (○ from chronic gastritis, ◍ from patients who later developed gastric cancer, and ● from gastric cancer patients), along with that in culture broth from strain 43504 (△). Bars indicate the median value of each group. The difference between gastritis patients and gastric cancer patients was statistically significant ($p = 0.004$).

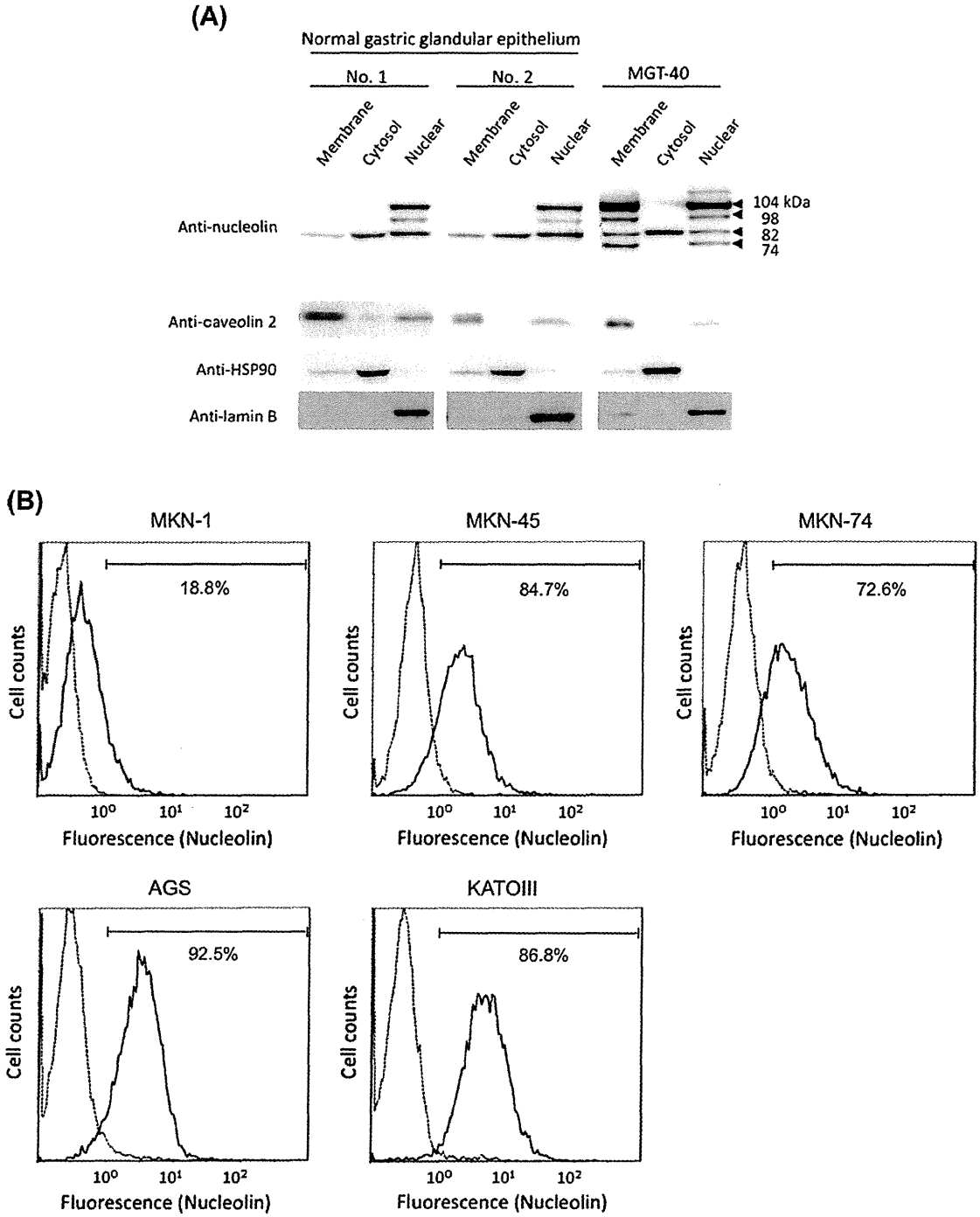

Fig. 3. Localization of nucleolin on the cell surface in gastric cancer cells, but not in normal gastric glandular epithelium. (A) Subcellular localization of nucleolin analyzed by cell fractionation. In normal gastric glandular epithelium, nucleolin was not detected in membrane fraction, but significant amounts of nucleolin were found in membrane fraction of MGT-40 cells by Western blotting with anti-nucleolin antibody. Antibodies of caveolin 2, HSP90 and lamin B were used for the controls with membrane, cytosol and nuclear fraction, respectively. (B) Detection of nucleolin on the cell surface of five gastric cancer cell lines by flow cytometry with anti-NUC295 antibody. Dotted line indicates control value incubated with pre-immune serum. % in each chart indicates percentage of cells with nucleolin on the cell surface in the region of the horizontal bar.

significant amounts of Tipα-FLAG were incorporated into the cells. Using immunoprecipitation with anti-nucleolin antibody, we found that incorporated Tipα-FLAG interacted with endogenous nucleolin in the cells, while incorporated del-Tipα-FLAG did not. Their differences in binding activity to nucleolin correlated well with activities of gene expression and transformation. Nucleolin was found to be present on the cell surface of MGT-40, THP-1 and human gastric cancer cell lines (MKN-1, MKN-45, MKN-74, AGS and KATOIII), although nucleolin is a well-known major protein in nucleoli [14,22]. In fact, significant amounts of nucleolin were present in membrane fraction as determined by cell fractionation analysis, although most of the nucleolin was still present in

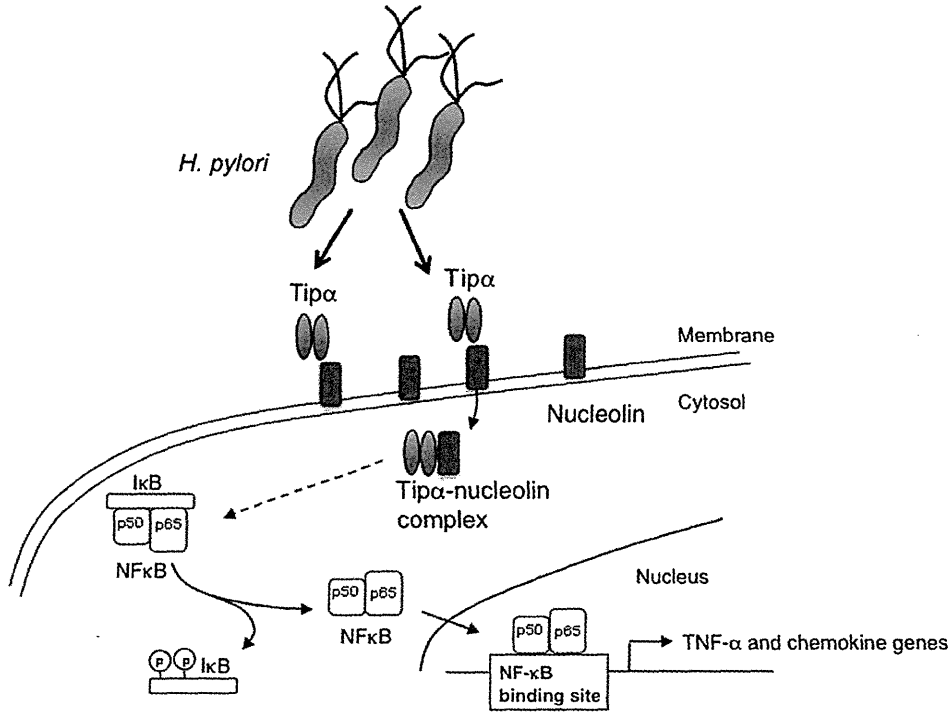

Fig. 4. Mechanism of gastric cancer development through interaction between Tipα and cell surface-nucleolin.

the nuclear fraction. However, normal epithelial cells of mouse glandular stomach did not contain any significant amounts of nucleolin in the membrane fraction (Fig. 3A). The localization of nucleolin on the cell surface was also determined by flow cytometry using anti-nucleolin (anti-NUC295) antibody. As shown in Fig. 3B, all five human gastric cancer cell lines have nucleolin on their cell surface, although the amounts varied [22].

Nucleolin on the cell surface is a glycoprotein containing *N*- and *O*-glycans, and the *N*-glycosylation of nucleolin determines the localization on cell surface [34,35]. Treatment with tunicamycin, an inhibitor of the *N*-linked glycosylation of protein, reduced the amounts of nucleolin on the cell surface as determined by flow cytometry [14]. Moreover, treatment with tunicamycin reduced incorporation of Tipα into MGT-40 cells, resulting in inhibition of *TNF-α* gene expression by Tipα. Together, these results strongly suggest that nucleolin on the cell surface is a specific receptor of Tipα, and that it shuttles Tipα from membrane to cytosol, and finally induces *TNF-α* gene expression by activation of NF-κB.

Furthermore, carcinogenic activity of Tipα is induced in cooperation with *v-H-ras* gene, as reported previously [11,23]. It has been shown that nucleolin interacts with both H-Ras protein and EGF receptor (EGFR) in cancer cells, and that nucleolin complex with mutated H-Ras protein (G12 V) and EGFR synergistically enhances anchorage-independent cell growth [36].

6. Tipα and nucleolin as new molecular targets for prevention and treatment of gastric cancer

It is now well understood that cell surface nucleolin serves as a receptor for numerous ligands, including lactoferrin, midkine, endostatin, human immunodeficiency virus (HIV), and human respiratory syncytial virus (RSV) [19,20,37–40], and that bovine lactoferrin performs antibacterial activity against *H. pylori in vitro* and suppresses its colonization in humans [41,42]. This suggests that lactoferrin inhibits Tipα activity by inhibiting the interaction between Tipα and nucleolin, resulting in inhibition of *H. pylori* infection. The results suggest that ligands, which bind to nucleolin and have anticancer activity, could be potentially used for prevention and treatment of gastric cancer in humans. For example, AS1411 is an anti-cancer DNA aptamer of 26-mer unmodified guanine-rich oligonucleotide, and it specifically binds to nucleolin [43,44]. AS1411 is now in phase II clinical trials in treatments for acute myeloid leukemia and renal cell carcinoma. In addition, it is important to note that AS1411 dose-dependently inhibited growth of gastric cancer cell lines in the order of MKN-45 > KATO-III > AGS > MKN-74 > MKN-1, with the order of the cell lines corresponding to the amount of cell surface nucleolin they contain [22]. There are additional reports showing that immunization via intranasal route with CpG + Tipα protein and CpG + del-Tipα protein reduced colonization of *H. pylori* in C57BL/6 mice, associated with higher levels of IFN-γ and IL-10 in the gastric mucosa [45]. Vaccination with Tipα or with del-Tipα would potentially be an effective way to prevent *H. pylori* infection, and probably gastric cancer. Thus, Tipα and cell-surface nucleolin are possible new targets in the prevention of *H. pylori* infection and gastric cancer development in humans.

7. Conclusion

Based on the observations that TNF-α is a master cytokine for tumor promotion in human cancer development, we have proposed a new carcinogenic process of *H. pylori* that is mediated through the interaction between Tipα and cell surface nucleolin (Fig. 4). Since normal mouse gastric epithelial cells do not have nucleolin on the cell surface, localization of nucleolin on the cell surface may be a limiting factor that is induced in very early stage of carcinogenesis, such as the initiation stage. Cells with nucleolin on cell surface become more sensitive to Tipα, associated with tumor promoting activity. Thus we believe that cell surface nucleolin is one of the key determinants in a host factor for development of gastric cancer by *H. pylori* infection.

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STUDY PROTOCOL Open Access

A randomized phase II trial to elucidate the efficacy of capecitabine plus cisplatin (XP) and S-1 plus cisplatin (SP) as a first-line treatment for advanced gastric cancer: XP ascertainment vs. SP randomized PII trial (XParTS II)

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Abstract

Background: On the basis of international clinical trials, capecitabine plus cisplatin (XP) as a first-line treatment of advanced gastric cancer is considered a global standard regimen. However, the usefulness of XP as compared with S-1 plus cisplatin (SP), which is considered standard therapy in Japan, has not yet been assessed.

Methods/design: This is a multicenter randomized phase II trial to elucidate the efficacy of XP as compared with SP for first-line treatment of advanced gastric cancer. Patients with unresectable metastatic or recurrent gastric cancer, 20–74 years of age and human epidermal growth factor 2 (HER2)-negative status, will be assigned in a 1:1 ratio to receive either S-1 40 mg/m^2 bid for 21 days plus cisplatin 60 mg/m^2 (day 8) every 5-week cycle or capecitabine 1000 mg/m^2 bid for 14 days plus cisplatin 80 mg/m^2 (day 1) every 3-week cycle. Patients will be also asked to the analysis of tumor tissues for translational investigations. The Primary endpoint is progression-free survival and secondary endpoints are overall survival, time to treatment failure, tumor response rate and safety. These comparisons will also be evaluated in terms of biomarkers. Planned sample size is 100 (50 in each arm), which is appropriate for this trial.

Discussion: Fluoropyrimidine plus cisplatin combination is the standard regimen of the first line treatment for advanced gastric cancer. Both S-1 and capecitabine are the prodrug of 5-FU but differ from their process of metabolism. Result of this trial and translational research will provide the important clues to prepare the individualized therapy for advanced gastric cancer in the near future.

Trial registration: ClinicalTrials.gov Identifier NCT01406249

Keywords: Biomarker, Capecitabine, Cisplatin, Clinical trial, Gastric cancer, S-1

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Background

Gastric cancer is the fourth most common malignancy in the world (988 602 cases in 2008, 7.8% of total) and the second leading cause of cancer death (737 419 deaths, 9.7% of total) [1]. For the treatment of advanced or recurrent gastric cancer (AGC), the most commonly used regimens are combination chemotherapy consisting of a fluoropyrimidine (5-fluorouracil or oral fluoropyrimidine) plus a platinum agent with or without docetaxel or anthracyclines [2-6].

S-1 is an oral anticancer drug composed of the 5-fluorouracil (5-FU) prodrug tegafur and two 5-FU modulators; it has achieved high response rates in patients with gastric cancer in phase II studies [7,8]. In a phase III trial (SPIRITS trial) that compared S-1 alone to S-1 plus cisplatin (SP), SP showed a significantly longer overall survival (OS; 13 months vs. 11 months; HR = 0.77, 95% CI 0.61–0.98, p = 0.04) and longer progression-free survival (PFS; 6.0 months vs. 4.0 months; HR = 0.57, 95% CI 0.44–0.73, p < 0.0001) [4]. Therefore, SP is now considered to be one of the standard first-line regimens for AGC in Japan.

Capecitabine is also an oral fluoropyrimidine, which is metabolized primarily in the liver and converted in tumor tissues to 5-FU by the enzyme thymidine phosphorylase (TP), which is associated in higher concentrations in tumor cells than in normal cells [9]. Kang and colleagues evaluated the non-inferiority of capecitabine plus cisplatin (XP) compared with 5-FU plus cisplatin (FP). The median PFS showed significant non-inferiority (5.6 months vs. 5.0 months; HR = 0.81, 95% CI 0.63–1.04, P < 0.001) [5]. On the basis of these results, XP is now considered one of the standard treatments of AGC [10], and XP was adopted as the reference arm in two recent global studies of molecular targeting agents [11,12]. However, data is scarce with respect to XP treatment in Japanese patients, and also the usefulness of XP as compared with SP has not yet been assessed.

As another issue, these 2 types of oral fluoropyrimidine show some different characteristics in the mechanisms of their antitumor effect. A subset analysis of the FLAGS trial showed that S-1 seemed to be better than 5-FU in the subgroup with diffuse-type gastric cancer [6]. This result was consistent with the results of a subset analysis of the JCOG9912 trial, which showed that S-1 was better than 5-FU in patients with diffuse-type gastric cancer or with gastric cancer associated with high dihydropyrimidine dehydrogenase (DPD), with diffuse-type tumors associated more commonly than intestinal type with high DPD [13]. This result was expected, since S-1 consists of tegafur, otastat potassium, and gimestat which is a potent competitive inhibitor of DPD. Capecitabine is transformed to 5-FU in several steps, to be finally converted by TP as above [9]. A phase II trial in Japan showed that response rate (RR) was significantly higher (Fisher's exact test, $p = 0.028$) in patients with TP-positive and DPD-negative tumors (60%, 6/10) than in the remaining patients (13%, 2/15) [14]. In contrast, high expression of TP is reported to be negatively associated with efficacy of 5-FU or S-1 in gastric cancer [15,16].

On the basis of the above reports, histological type (diffuse or intestinal) and biomarkers (TP, DPD, and others) may be candidates to select whether S-1 or capecitabine be used for each patient, although validation with a randomized study is necessary. We planned the current clinical trial to elucidate the efficacy of XP and SP for the first-line treatment of AGC. This comparison will be also evaluated in terms of several biomarkers.

Method/design

Study objective

This randomized phase II trial is planned to elucidate the efficacy of SP and XP and also to explore predictive or prognostic biomarkers with additional research. This trial protocol has been approved by the Institutional Review Board (IRB) of each participating institution and the Kanagawa Cancer Center.

Study endpoints

Primary endpoint is PFS and secondary endpoints are OS, RR, time to treatment failure (TTF), and incidence of adverse events (safety).

Eligibility criteria

Inclusion criteria

(i) Histologically confirmed gastric adenocarcinoma with unresectable metastatic or recurrent disease
(ii) Lesions confirmed by imaging no more than 28 days before registration (not required for measurable lesions as defined in RECIST version 1.1)
(iii) No previous chemotherapy or radiotherapy. However, prior adjuvant chemotherapy is allowed if more than 6 months has passed since the end of adjuvant chemotherapy
(iv) Eastern Cooperative Oncology Group (ECOG) Performance Status of 0, 1, or 2
(v) Life expectancy of at least 3 months after registration
(vi) Written informed consent
(vii) Between the ages of 20 and 74 years at the time informed consent is obtained
(viii) Adequate major organ function including:
(a) Neutrophil count: ≥1500/mm^3
(b) Platelet count: ≥10.0 × 10^4/mm^3
(c) Hemoglobin: ≥9.0 g/dL
(d) AST, ALT: ≤2.5 × upper limit of normal (ULN) in each institution (≤5 times in cases of metastases to liver)
(e) ALP: ≤2.5 × ULN in each institution (≤5 times in cases of metastases to liver, and ≤10 times in cases of metastases to bone)

(f) Total bilirubin: ≤1.5 × ULN in each institution
(g) Creatinine clearance: ≥60 mL/min (as estimated by Cockcroft-Gault equation)

Exclusion criteria

(i) HER2- positive status
(ii) Previous history of fluoropyrimidine therapy within 6 months prior to registration
(iii) Previous treatment with platinum agents within 12 months prior to registration
(iv) Previous treatment with cisplatin more than total dose of 120 mg/m^2
(v) Previous history of serious hypersensitivity to fluoropyrimidines or platinum agents
(vi) Previous history of adverse reactions suggestive of dihydropyrimidine dehydrogenase (DPD) deficiency
(vii) More than 1 cancer at the same time or more than 1 cancer at different times separated by a 5-year disease-free interval. However, multiple active cancers do not include carcinoma *in situ* or skin cancer which is determined to have been cured as a result of treatment.
(viii) Obvious infection or inflammation (pyrexia ≥38.0 °C)
(ix) Active hepatitis
(x) Heart disease that is serious or requires hospitalization, or history of such disease within the past year
(xi) Having a complication that is serious or requires hospitalization (intestinal paralysis, intestinal obstruction, interstitial pneumonia or pulmonary fibrosis, poorly controlled diabetes mellitus, renal failure, liver disorders, or hepatic cirrhosis)
(xii) Being treated or in need of treatment with flucytosine, phenytoin, or warfarin potassium
(xiii) Chronic diarrhea (watery stools or ≥4 times/day)
(xiv) Active gastrointestinal bleeding
(xv) Body cavity fluids requiring drainage or other treatment
(xvi) Clinical suspicion or previous history of metastasis to brain or meninges
(xvii) Women who are pregnant, breastfeeding, or potentially (hoping to become) pregnant
(xviii) Unwillingness to practice contraception
(xix) Poor oral intake
(xx) Psychiatric disorders which are being, or may need to be, treated with psychotropics
(xxi) Otherwise determined by investigators or site principal investigators to be unsuitable for participation in study

Registration

Physicians or coordinators will send a Case Registration Form to the data center (Epidemiological and Clinical Research Information Network, ECRIN) with all the required items filled out. Enrollment has started from July 2011.

Startification

Eligible patients will be randomized to either Arm-A (SP treatment) or Arm-B (XP treatment) by dynamic allocation via a centralized randomization method using 5 stratification factors as balancing variables:

(i) baseline ECOG Performance Status (0–1/2)
(ii) measurable lesion (yes/no)
(iii) prior adjuvant chemotherapy (yes/no)
(iv) histopathological classification (intestinal/diffuse)
(v) institution.

Statistical analysis

PFS has been set as the primary endpoint and is defined as the time from date of registration until the date that progression is determined or the date of death for any reason, whichever is sooner. "Progression" will be evaluated on the basis of Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1 [17]. More information about the definition of PFS and Progression are pre-specified (Table 1). T1

The primary objective of this trial is to evaluate the PFS of SP and XP as the first-line treatment for advanced gastric cancer. The 24-week progression-free rate (PFR) will be estimated for each group, calculating point estimates and 2-sided 90% confidence intervals. The 2-sided 90% confidence interval of the difference between the 2 groups will be also estimated. Exploratory analysis will be done to test the null hypothesis that PFS is equal in both groups. Cumulative PFS curves will be constructed as time-to-event plots by the Kaplan-Meier method.

With respect to secondary endpoints, efficacy endpoints OS and TTF will be evaluated according to the method of analysis of the primary endpoint. Overall response rate (RR) is defined as the proportion of patients with complete response (CR) or partial response (PR) by RECIST out of the patients with measurable lesions, and the chi-square test will be used to compare the 2 groups. The 2-sided 95% confidence interval of the difference between the 2 groups will also be estimated. For the analysis of safety, Fisher's exact test will be used if necessary, and the exact confidence intervals for the binomial distribution will be estimated.

Sample-size calculation

Assuming a threshold 24-week PFR of 40% and an expected 24-week PFR of 55% (clinically promising), and a 1.5-year registration period and a 1.5-year follow-up period, 49 patients are required in each group to ensure a 1-sided alpha of 5% and statistical power of 90%. Assuming that the 24-week PFR of the biomarker-positive (any FU-related enzyme or expression of intestinal type) population

Table 1 Definition of PFS and progression

Definition of PFS and progression are predefined as below
1.) PFS will be determined as the time from the date of registration until the date that progression is determined or the date of death for any reason, whichever is sooner.
2.) "Progression (PD)" means both PD confirmed by routine diagnostic imaging in each course and PD confirmed by as-needed diagnostic imaging in the case that there is clinical suspicion of PD. In the latter case, it is preferable that there is at least objective evidence.
3.) When progression is determined based on diagnostic imaging, the date of progression will be the date on which imaging is assessed. When clinical progression is first determined independently of diagnostic imaging, and then later objectively determined on the basis of diagnostic imaging, the date of progression will be back-dated to the date of determination of clinical progression. If no objective evidence is obtained, it will be treated as a censoring event in the formal analysis, and sensitivity analysis will be also conducted as if this were PD.
4.). When considering tumor regrowth and determining PD according to RECIST, it is considered a PD as PFS event regardless of tumor diameter. But even if it is decided as PD according to RECIST, investigators can continue the protocol treatment if they consider continued treatment to be beneficial to the patient.
5.) If treatment discontinuation is needed due to symptomatic deterioration without any objective evidence at that time, it is reported as "symptomatic deterioration". Investigators should endeavor to obtain objective evidence of the progression even after discontinuation of treatment. In this case, the event shall be judged to be clinical PD and handled as mentioned in 2) above. When progression is determined on the basis of diagnostic imaging, the date of progression will be back-dated to the date of diagnosis of symptomatic deterioration.
6.) Survivors for whom progression has not been determined will be censored based on the last date on which the absence of progression was clinically confirmed (the last day that PFS was confirmed).
7.) Cases of discontinuation of protocol treatment because of toxicity or patient refusal, even if another therapy is added as a post-treatment, will be censored at the date of discontinuation or the date that post-treatment was started.
8.) In cases where progression is diagnosed on the basis of imaging, the event will be determined based not on evaluation dates where the result is "suspected" on imaging but on a subsequent evaluation date where progression is "confirmed" on imaging.
9.) Secondary cancer (multiple cancers in metachronous) will not be regarded as either an event or censored.

in the SP arm is 45%, and the risk reduction rate in the XP arm is 40%, 46 patients in total are needed to ensure a 2-sided alpha of 10% and statistical power of 70%. Under the hypothesis that the targeted biomarker-positive population is 50%, 92 patients in total are required. Considering the likelihood of some ineligible cases in the whole setting outlined above, the total sample size is set to 100. A following Phase III study will be designed for both randomized comparison and biomarker-oriented comparison of XP and SP (4 groups).

Treatment program

Patients who allocated SP will be treated with S-1 and cisplatin every 5-week cycle. S-1 will be administered orally at a dose of 40 mg/m^2 twice-daily (equivalent to a total daily dose of 80 mg/m^2) for 3 weeks (day 1 to 21). Cisplatin 60 mg/m^2 on day 8 of each cycle will be given by intravenous infusion over 2 hours. On the other hand, patients who allocated XP will be treated with capecitabine and cisplatin every 3-week cycle. Capecitabine will be administered orally at a dose of 1000 mg/m^2 twice-daily (equivalent to a total daily dose of 2000 mg/m^2) for 2 weeks (day 1 to 14). Cisplatin 80 mg/m^2 on day 1 of each cycle will be given by intravenous infusion over 2 hours.

Treatment continuation is intended until disease progression or unacceptable toxicity. If treatment continuation with cisplatin is determined to be unfeasible before any progression is confirmed, continuously monotherapy of S-1 or capecitabine will be continued until PD.

Follow-up

During treatment under this protocol, patients will have a physical check-up and a blood examination before every drug administration. PFS and RR will be monitored by using abdominal CT or MRI every 6 weeks and by measuring levels of tumor markers CEA and CA19-9.

Translational research project

Translational research will be conducted to elucidate the clinical utility of the following biomarkers. These biomarkers will be analyzed Immunohistochemistry (IHC) and mRNA expression by using tissue specimen. Tumor tissue samples from primary lesions and/or biopsy material will be collected and centralized assessment.

(i) Immunohistochemistry (IHC): Expression of TP, DPD, ERCC1, Ki67, LGALS4, and CDH17
(ii) mRNA: Expression of TP, DPD, thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), and excision repair cross-complementation group1 (ERCC1)

Discussion

Recently, molecular target drugs has resulted in the opportunity to provide individualized treatment in the field of AGC. Especially in patients with HER2-positive AGC (defined as assessed by IHC 3+ on a scale of 0 to 3+, and/or fluorescence in-situ hybridization; FISH, *HER2*:CEP17 ratio ≥2.0), ToGA study showed that adding trastuzumab was significantly improved overall survival comparing with standard chemotherapy consists of cytotoxic drugs [11]. This study excludes HER2-positive gastric cancer since these patients should be recommended trastuzumab containing regimen. The individualized treatment for cytotoxic agents also needs to be developed to have more effect and less toxicity.

This is the first study to compare two standard regimens for AGC. Additionally, the translational research is

performed to explore the biomarker for chemo-sensitivity and make the individualized treatment possible. When the difference of treatment is found in efficacy or safety from this analysis, we will conduct a phase III trial to examine the possibility of individualized treatment. We believe the result of this study will play the important role to prepare the individualized therapy for advanced gastric cancer in the near future.

Competing interests

All authors declare that they have no competing interest.

Authors' contributions

AT drafted the manuscript and wrote the original protocol for the study. All authors participated in the design of the study. SM performed the statistical analysis. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Effect of RECIST revision on classification of target lesions and overall response in advanced gastric cancer patients

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Abstract
Background The Response Evaluation Criteria in Solid Tumors (RECIST) was revised in 2009, based on a large dataset of 6512 patients from 16 trials. However, no gastric cancer patients were included in those data. The purpose of this study was to clarify the difference between RECIST version 1.0 and version 1.1 in advanced gastric cancer.
Methods From 2004 to 2009, 129 consecutive patients with advanced gastric cancer received S-1 plus cisplatin as first-line treatment at the National Cancer Center Hospital East. Ninety-seven of these patients who had had baseline and post-treatment computed tomography scans performed were included in this study. Measurements of tumors were conducted retrospectively.
Results At the baseline of first-line chemotherapy, 172 lymph nodes in 54 patients were considered to be candidate target lesions by RECIST version 1.0. However, only 38 % of the lymph nodes were classified as target lesions by RECIST version 1.1, with 47 % classified as non-target lesions and 15 % classified as non-pathological. By RECIST version 1.0, the proportion of patients with target lesions at the baseline of first-line chemotherapy was 67 % (65/97), and this percentage was significantly reduced according to RECIST version 1.1 (53 %; 51/97) (McNemar's exact test, $P < 0.001$). The findings at the baseline of second-line chemotherapy were similar (reduced from 62 to 49 %; McNemar's exact test, $P = 0.002$). Overall response rates of first-line chemotherapy were 52 % (34/65) according to RECIST version 1.0 and 55 % (28/51) according to version 1.1.
Conclusions The revision of RECIST significantly reduced the proportion of patients classified with target lesions at the baselines of first-line and second-line chemotherapies. No obvious difference in overall response rates was observed.

Keywords RECIST · Gastric cancer · Target lesion

Introduction

The Response Evaluation Criteria in Solid Tumors (RECIST) have been widely used as standard criteria to evaluate the objective responses of chemotherapy, and the RECIST version 1.0 were revised to version 1.1 in 2009 [1, 2]. Major changes in the revised RECIST version 1.1 are as follows. (1) The number of lesions required to assess tumor burden has been reduced from a maximum of 10 to 5 in total, and from a maximum of 5 to 2 per organ. (2) Lymph nodes with a ≥15 mm short axis are considered measurable as target lesions, those with a ≥10 to <15 mm short axis are considered assessable as non-target lesions, and those with a <10 mm short axis are considered non-pathological. (3) Additionally, the definitions of complete response (CR) and progressive disease (PD) were revised. In the response criteria for CR, especially in regard to lymph node evaluation, the requirement for the disappearance of all lesions was revised to any pathological lymph nodes having a reduction in the short axis of <10 mm. In the response criteria for PD,

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RECIST version 1.1 requires a 5 mm absolute increase, in addition to a target lesion with a 20 % increase in the sum of the diameters, to avoid a clinically inappropriate diagnosis of PD when the total sum of lesion diameters is very small [1].

The revision, RECIST 1.1, was based on a large dataset (RECIST data warehouse) of 6512 patients from 16 clinical trials, consisting of 7 breast cancer trials, 4 lung cancer trials, 2 colorectal cancer trials, 2 renal cell carcinoma trials, and 1 gastrointestinal stromal tumor trial, performed between 1993 and 2005 [1, 2]. However, no gastric cancer patients were included in those data.

While surgery remains the only possible cure in patients with early-stage gastric cancer, palliative chemotherapy is the mainstay for patients with inoperable advanced or recurrent cancer. Although there is no globally accepted standard chemotherapy regimen, a fluoropyrimidine plus a platinum agent with or without epirubicin or docetaxel are the protocols used for advanced gastric cancer patients [3]. Based on several randomized controlled trials, a combination of tegafur, gimeracil, and potassium oxonate (S-1) plus cisplatin (CDDP) is widely used and accepted as standard chemotherapy in Japan [4–6].

The purpose of this study was to clarify the differences between RECIST version 1.0 and version 1.1 in terms of the proportions of patients classified with target lesions at the baselines of first- and second-line chemotherapies and the overall response rate (ORR) in advanced gastric cancer patients who received S-1 plus CDDP as first-line chemotherapy.

Patients and methods

From 2004 to 2009, 129 consecutive patients with advanced gastric cancer received S-1 plus CDDP as first-line treatment at the National Cancer Center Hospital East. S-1 (40–60 mg depending on the patient's body surface area as follows: <1.25 m^2, 40 mg; ≥ 1.25 m^2 and <1.5 m^2, 50 mg; and ≥ 1.5 m^2, 60 mg) was given orally twice daily for 3 consecutive weeks and CDDP was given intravenously at a dose of 60 mg/m^2 on day 8, followed by a 2-week rest period, within a 5-week cycle. Of all 129 patients, 97 patients who met the following criteria were included in this study: histologically confirmed unresectable and recurrent adenocarcinoma of the stomach, having no other malignancy, no history of chemotherapy or radiation therapy except for adjuvant chemotherapy, and tumor assessment by computed tomography (CT) scans performed in our hospital at baseline (within 28 days before the start of treatment) and post-treatment.

All CT scans were performed on a helical CT scanner with intravenous administration of contrast materials, and the slice thickness was 5 mm. The post-treatment CT scans were performed after every 2 cycles of S-1 plus CDDP. The CT image data were directly displayed on monitors and tumor measurements were performed with electronic calipers. We reviewed each patient's medical records and measured tumor size retrospectively using RECIST version 1.0 and version 1.1. Two medical oncologists (N.F. and E.N.) reviewed all CT images independently of the attending physicians. First, E.N. evaluated all CT images based on RECIST versions 1.0 and 1.1. N.F. then reviewed the results. If an inter-observer difference was present, the final judgment was made after sufficient discussion. The overall response was evaluated without interval confirmation.

The differences in proportions of patients with target lesions between the two RECIST versions were evaluated using McNemar's exact test. Corresponding 95 % confidence intervals (CIs) were also calculated, using the Clopper-Pearson method. All P values are two sided. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC, USA).

All data were collected retrospectively. The study was performed under an institutional review board waiver in accordance with the Japanese ethical guidelines for epidemiological research.

Results

The characteristics of the 97 patients are shown in Table 1. All 97 patients discontinued S-1 plus CDDP, for the following reasons: 88 patients because of PD, 5 because of adverse events, and 4 for other reasons. Of the 88 patients with PD after S-1 plus CDDP, 74 (84 %) received second-line chemotherapy.

One hundred seventy-two lymph nodes from 54 patients were considered to be candidate target lesions by RECIST version 1.0 at the baseline of the first-line chemotherapy. These lymph nodes were categorized into 3 groups according to the size of the short axis by RECIST version 1.1 as follows: <10 mm; ≥ 10 mm but <15 mm; and ≥ 15 mm (Table 2). According to RECIST version 1.1, only 38 % (66/172) of the lymph nodes were classified as target lesions, 47 % (80/172) were classified as non-target lesions and 15 % (26/172) were classified as non-pathological lesions.

Target lesions at the baselines of the first- and second-line chemotherapies, classified according to RECIST version 1.0 and version 1.1, are summarized in Table 3. The proportion of patients with a target lesion at the baseline of the first-line chemotherapy was 67 % (65/97; 95 % CI 57–76 %) by RECIST version 1.0, and the proportion was reduced to 53 % (51/97; 95 % CI 42–63 %) when classified according to RECIST version 1.1. This reduction was statistically significant (McNemar's exact test, $P < 0.001$).