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

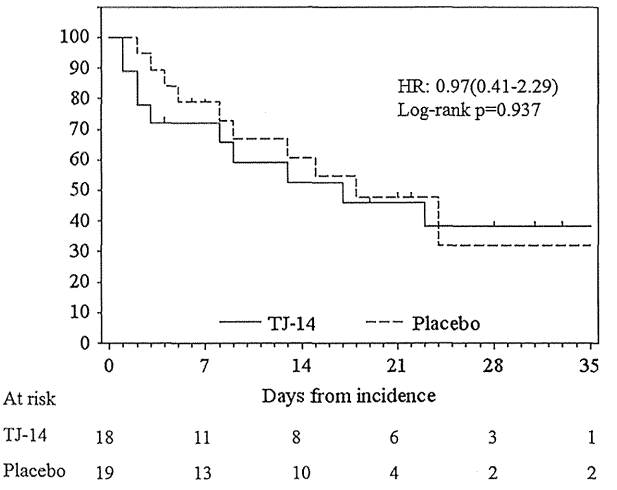

Fig. 1 Duration of ≧grade 2 chemotherapy-induced oral mucositis in the treatment group

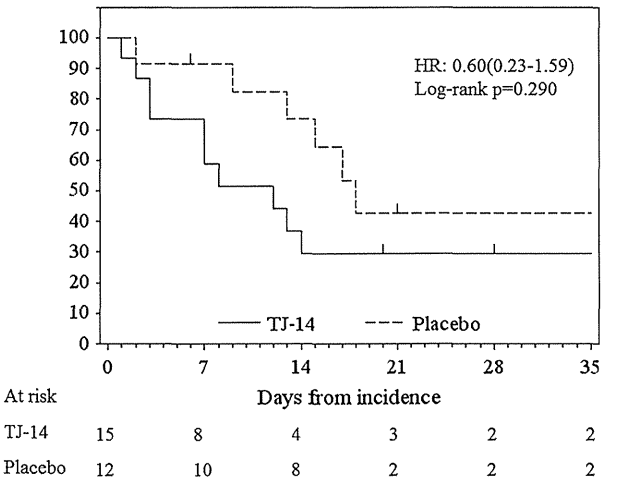

Fig. 2 Duration of any grade of chemotherapy-induced oral mucositis in the patients who developed grade 1 oral mucositis during the screening cycle

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.

Acknowledgments This study was supported in part by a nonprofit organization, the Epidemiological & Clinical Research Information Network (ECRIN). The authors express their sincere gratitude to Ms. Mai Hatta for her excellent data management in this study.

Conflict of interest None declared.

References

1. Ohtsu A, Yoshida S, Saijo N (2006) Disparities in gastric cancer chemotherapy between the East and West. J Clin Oncol 24:2188–2196
2. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN et al (2001) Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 345:725–730
3. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M et al (2006) Perioperative chemotherapy versus surgery alone for resectable gastro esophageal cancer. N Engl J Med 355:11–20
4. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A et al (2007) Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 357:1810–1820
5. Bang YJ, Kim YW, Yang HK, Chung HC, Park YK, Lee KH et al (2012) Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. Lancet 28(379):315–321
6. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M et al (2008) S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 9:215–221
7. Ajani JA, Rodriguez W, Bodoky G, Moiseyenko V, Lichinitser M, Gorbunova V et al (2010) Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: the FLAGS trial. J Clin Oncol 28:1547–1553
8. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A et al (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. Lancet 376:687–697
9. Scardina GA, Pisano T, Messina P (2010) Oral mucositis. Review of literature. N Y State Dent J 76:34–38
10. Napenas JJ, Shetty KV, Streckfus CF (2007) Oral mucositis: review of pathogenesis, diagnosis, prevention, and management. Gen Dent 55:335–344
11. Scully C, Sonis S, Diz PD (2006) Oral mucositis. Oral Dis 12:229–241
12. Logan RM, Gibson RJ, Sonis ST, Keefe DM (2007) Nuclear factor-kappaB (NF-kappaB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy. Oral Oncol 43:395–401
13. Clarkson JE, Worthington HV, Eden OB (2003) Interventions for preventing oral mucositis for patients with cancer receiving treatment. Cochrane Database Syst Rev (3):CD000978
14. Jensen SB, Jarvis V, Zadik Y, Barasch A, Ariyawardana A, Hovan A et al (2013) Systematic review of miscellaneous agents for the management of oral mucositis in cancer patients. Support Care Cancer. PubMed PMID: 23900593 (Epub ahead of print)
15. Kono T, Kanematsu T, Kitajima M (2009) Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet? Surgery 146:837–840
16. Hibi S, Ina K, Furuta R, Kataoka T, Kojima S, Kawai M (2009) Clinical effects of Hange-shashin-to on combination therapy of S-1/irinotecan against the for patients with metastatic gastric and colorectal cancer. Gan To Kagaku Ryoho 36(9):1485–1488
17. Mori K, Kondo T, Kamiyama Y, Kano Y, Tominaga K (2003) Preventive effect of Kampo medicine (Hangeshashin-to) against irinotecan-induced diarrhea in advanced non-small-cell lung cancer. Cancer Chemother Pharmacol 51(5):403–406
18. Kono T, Satomi M, Chisato N, Ebisawa Y, Suno M, Asama T, Karasaki H, Matsubara K, Furukawa H (2010) Topical application of hangeshashinto (TJ-14) in the treatment of chemotherapy-induced oral mucositis. World J Oncol 1(6):232–235
19. Nakazono Y, Ara T, Fujinami Y, Hattori T, Wang PL (2010) Preventive effects of a kampo medicine, hangeshashinto on inflammatory responses in lipopolysaccharide-treated human gingival fibroblasts. J Hard Tissue Biol 19(1):43–50
20. Kase Y, Hayakawa T, Aburada M, Komatsu Y, Kamataki T (1997) Preventive effects of Hange-shashin-to on irinotecan hydrochloride-caused diarrhea and its relevance to the colonic prostaglandin E2 and water absorption in the rat. Jpn J Pharmacol 75(4):407–413
21. Kase Y, Saitoh K, Yuzurihara M, Ishige A, Komatsu Y (1998) Effects of Hange-shashin-to on cholera toxin-induced fluid

secretion in the small intestine of rats. Biol Pharm Bull 21(2):117–120
22. Kase Y, Saitoh K, Makino B, Hashimoto K, Ishige A, Komatsu Y (1999) Relationship between the antidiarrhoeal effects of Hange-Shashin-To and its active components. Phytother Res 13(6):468–473
23. Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN (2007) The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine 14(2–3):123–128
24. Pan MH, Hsieh MC, Hsu PC, Ho SY, Lai CS, Wu H, Sang S, Ho CT (2008) 6-Shogaol suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. Mol Nutr Food Res 52(12):1467–1477
25. Nievergelt A, Marazzi J, Schoop R, Altmann KH, Gertsch J (2011) Ginger phenylpropanoids inhibit IL-1beta and prostanoid secretion and disrupt arachidonate-phospholipid remodeling by targeting phospholipases A2. J Immunol 187(8):4140–4150
26. van Breemen RB, Tao Y, Li W (2011) Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). Fitoterapia 82(1):38–43
27. Nakahata N, Tsuchiya C, Nakatani K, Ohizumi Y, Ohkubo S (2003) Baicalein inhibits Raf-1-mediated phosphorylation of MEK-1 in C6 rat glioma cells. Eur J Pharmacol 461(1):1–7
28. Chi YS, Kim HP (2005) Suppression of cyclooxygenase-2 expression of skin fibroblasts by wogonin, a plant flavone from *Scutellaria radix*. Prostaglandins Leukot Essent Fatty Acids 72(1):59–66
29. Wakabayashi I, Yasui K (2000) Wogonin inhibits inducible prostaglandin E (2) production in macrophages. Eur J Pharmacol 406(3):477–481
30. Feng AW, Yu C, Mao Q, Li N, Li QR, Li JS (2011) Berberine hydrochloride attenuates cyclooxygenase-2 expression in rat small intestinal mucosa during acute endotoxemia. Fitoterapia 82(7):976–982
31. Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, Kim WS, Kim JB (2009) Berberine suppresses proinflammatory responses through AMPK activation in macrophages. Am J Physiol Endocrinol Metab 296(4):E955–E964
32. Feng AW, Gao W, Zhou GR, Yu R, Li N, Huang XL, Li QR, Li JS (2012) Berberine ameliorates COX-2 expression in rat small intestinal mucosa partially through PPARgamma pathway during acute endotoxemia. Int Immunopharmacol 12(1):182–188
33. Lalla RV, Peterson DE (2005) Oral mucositis. Dent Clin North Am 49:167–184
34. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M et al (2004) Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. Cancer 100:1995–2025
35. Keefe DM, Schubert MM, Elting LS, Sonis ST, Epstein JB, Raber-Durlacher JE et al (2007) Updated clinical practice guidelines for the prevention and treatment of mucositis. Cancer 109:820–831
36. Coleman RE, Biganzoli L, Canney P, Dirix L, Mauriac L, Chollet P et al (2006) A randomised phase II study of two different schedules of pegylated liposomal doxorubicin in metastatic breast cancer (EORTC-10993). Eur J Cancer 42:882–887
37. Elting LS, Cooksley C, Chambers M, Cantor SB, Manzullo E, Rubenstein EB (2003) The burdens of cancer therapy. Clinical and economic outcomes of chemotherapy-induced mucositis. Cancer 98:1531–1539
38. Kase Y, Hayakawa T, Ishige A, Aburada M, Komatsu Y (1997) The effects of Hange-shashin-to on the content of prostaglandin E2 and water absorption in the large intestine of rats. Biol Pharm Bull 20:954–957
39. Kase Y, Saitoh K, Ishige A, Komatsu Y (1998) Mechanisms by which Hange-shashin-to reduces prostaglandin E2 levels. Biol Pharm Bull 21:1277–1281
40. Nakazono Y, Ara T, Fujinami Y, Hattori T, Wang PL (2010) Preventive effects of a Kampo, Hangeshashinto on inflammatory responses in lipopolysaccharide-treated human gingival fibroblasts. J Hard Tissue Biol 19:43–50

Ann Surg Oncol
DOI 10.1245/s10434-014-3615-8

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Accuracy of CT Staging of Locally Advanced Gastric Cancer after Neoadjuvant Chemotherapy: Cohort Evaluation within a Randomized Phase II Study

Takaki Yoshikawa, MD, PhD[1], Kazuaki Tanabe, MD[2], Kazuhiro Nishikawa, MD[3], Yuichi Ito, MD[4], Takanori Matsui, MD[5], Yutaka Kimura, MD, PhD[6], Shinichi Hasegawa, MD[1], Toru Aoyama, MD[1], Tsutomu Hayashi, MD[1], Satoshi Morita, PhD[7], Yumi Miyashita[8], Akira Tsuburaya, MD[9], and Junichi Sakamoto, MD[10]

[1]Department of Gastrointestinal Surgery, Kanagawa Cancer Center, Yokohama, Japan; [2]Department of Gastrointestinal Surgery, Hiroshima University, Hiroshima, Japan; [3]Department of Surgery, Osaka General Medical Center, Osaka, Japan; [4]Department of Gastroenterological Surgery, Aichi Cancer Center, Nagoya, Japan; [5]Department of Surgery, Aichi Cancer Center, Aichi Hospital, Okazaki, Japan; [6]Department of Surgery, NTT West Osaka Hospital, Osaka, Japan; [7]Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, Yokohama, Japan; [8]Data Center, Nonprofit Organization ECRIN, Okazaki, Japan; [9]Clinical Research Center, Shonan Kamakura General Hospital, Kamakura, Japan; [10]Tokai Central Hospital, Kakamigahara, Japan

ABSTRACT

Background. Accuracy of the radiologic diagnosis of gastric cancer staging after neoadjuvant chemotherapy remains unclear.

Methods. Patients enrolled in the COMPASS trial, a randomized phase II study comparing two and four courses of S-1 plus cisplatin and paclitaxel and cisplatin followed by gastrectomy, were examined. The radiologic stage was determined by using thin-slice computed tomography (CT) or multidetector low CT by following Habermann's method.

Results. A total of 75 patients registered in the COMPASS study who underwent surgical resection were examined in this study. The radiologic T and pathologic T stages were not significantly correlated ($p = 0.221$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 42.7, 10.7, and 46.7%, respectively. When patients were stratified according to the pathologic response of the primary tumor, the correlation was not significant in either the responders ($n = 32$, $p = 0.410$) or the nonresponders ($n = 43$, $p = 0.742$). The radiologic accuracy was 37.5% in the responders and 42.7% in the nonresponders. The radiologic N and pathologic N stages were significantly correlated ($p = 0.000$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 44, 29.3, and 26.7%, respectively. When stratifying the patients with measurable lymph nodes according only to the radiologic response, the correlation was significant in the nonresponders ($n = 23$, $p = 0.035$) but not in the responders ($n = 28$, $p = 0.634$). The radiologic accuracy was 39.3% in the responders and 52.1% in the nonresponders.

Conclusions. Restaging using CT after neoadjuvant chemotherapy for gastric cancer is considered to be inaccurate and unreliable. In particular, the radiologic T-staging determined after neoadjuvant chemotherapy should not be considered in clinical decision-making.

Gastric cancer is the second leading cause of cancer death worldwide, accounting for 736,000 deaths in 2008.[1] Complete surgical resection is essential for curing gastric cancer. Recent large phase III studies have demonstrated that multimodality treatment including surgery significantly improves the survival of locally advanced disease compared with surgery alone, postoperative adjuvant chemotherapy with S-1 in Japan, postoperative adjuvant chemotherapy with capecitabine plus oxaliplatin in Korea and the United States, and preoperative and postoperative chemotherapy with epirubicin, cisplatin, and fluorouracil in the United Kingdom.[2–8]

Neoadjuvant chemotherapy is a promising treatment for gastric cancer when considering intensive chemotherapy with a relatively toxic regimen.[2] Even with treatment

First Received: 19 October 2013

T. Yoshikawa, MD, PhD
e-mail: yoshikawat@kcch.jp

Published online: 05 March 2014

including D2 gastrectomy and adjuvant chemotherapy, the prognosis of stage III tumors is not satisfactory.[5] Neoadjuvant chemotherapy has been tested in several phase III trials in eastern Asia where D2 gastrectomy and adjuvant chemotherapy is a standard treatment.[2] After administering neoadjuvant chemotherapy, physicians must evaluate tumor progression and the response to treatment in order to continue or stop the chemotherapy and to assess resectability with respect to surgery and determine the most appropriate surgical procedure to fit the tumor stage considering the benefits and risks of surgery.

Endoscopic ultrasonography (EUS) and computed tomography (CT) are standard approaches for staging primary gastric cancer. The diagnostic accuracy of T-staging is 77.1 to 88.9% on CT and 65 to 92.1% on EUS, whereas that of N-staging is 51 to 71% on CT and 63 to 78% on EUS.[9,10] However, there are no reliable data with respect to restaging after neoadjuvant chemotherapy. Previously, several small studies demonstrated that preoperative EUS is inaccurate in patients who receive neoadjuvant chemotherapy.[11,12] Regarding CT, Park et al.[13] reported that the accuracy of T- and N-staging after neoadjuvant chemotherapy using CT is 57 and 37%, respectively. However, the sample size was only 38 in their study, and the evaluation criteria for assessing tumor depth were not defined. Moreover, the criteria for determining nodal metastasis were not optimized.

To evaluate the radiologic accuracy of restaging after neoadjuvant chemotherapy using CT, the present study was conducted as an exploratory analysis of a randomized phase II study that strictly defined primary staging, neoadjuvant chemotherapy, restaging after neoadjuvant chemotherapy, and the surgical procedures.

PATIENTS AND METHODS

Patients registered into the randomized phase II COMPASS trial who received gastrectomy with nodal dissection were examined in this study. The details of the COMPASS trial have been described in a previous article.[14] Briefly, the key eligibility criteria included T2–3/N+ or T4aN0 in cases of scirrhous or junctional tumors, T2–3 with nodal metastasis to the major branched artery, T4aN+ , T4b, paraaortic nodal metastases, or resectable minimal peritoneal metastases confirmed on laparoscopy. The use of staging laparoscopy was mandatory to diagnose peritoneal metastasis. The eligible patients were randomized to receive two courses of S-1 plus cisplatin, four courses of S-1 plus cisplatin, two courses of paclitaxel plus cisplatin, or four courses of paclitaxel plus cisplatin. The primary end point of the COMPASS trial is the 3-year overall survival rate and will recruit 60 to 80 subjects. This study was conducted in a cohort of consecutive patients recruited into the COMPASS trial.

Regarding the S-1 plus cisplatin regimen, S-1 (80 mg/m^2) was given orally twice daily for the first 3 weeks of a 4-week cycle, and cisplatin was given as an intravenous infusion of 60 mg/m^2 on day 8 of each cycle, as previously described.[15] With respect to the paclitaxel plus cisplatin regimen, paclitaxel (60 mg/m^2) and cisplatin (25 mg/m^2) were administered on days 1, 8, and 15 as one course repeated every 4 weeks.[16] The neoadjuvant chemotherapy was discontinued in cases of documented disease progression, unacceptable toxicity, or withdrawal of consent.

Two to six weeks after the completion of neoadjuvant chemotherapy or when the tumors progressed during treatment, the patients proceeded to surgery. R0 resection was achieved with gastrectomy and standard D2 lymphadenectomy.[17] Paraaortic nodal dissection or combined resection of a small portion of the peritoneum or adjacent organs was permitted for curative intent; however, more invasive procedures, such as pancreaticoduodenectomy or Appleby's surgery, were not. When macroscopically curative surgery was achieved, the protocol treatment was terminated.

The radiologic diagnosis of T and N was determined by using thin-slice CT with a 5- to 7-mm thickness or multidetector low CT by following Habermann's method.[18,19] T1 tumors were defined as tumors that could not be found on images or that had focal thickening of the inner layer with a visible outer layer of the gastric wall and a clear fat plane around the lesion. T2 tumors were defined as tumors with focal or diffuse thickening of the gastric wall with transmural involvement and a smooth outer border of the wall or only a few small linear strands of soft tissue extending into the fat plane involving less than one-third of the tumor extent. T3 tumors were defined as transmural tumors with obvious blurring of at least one-third of the tumor extent or wide reticular strands surrounding the outer border of the tumor. T4 tumors were defined as tumors with obliteration of the fat plane between the gastric tumor and the adjacent organ or invasion of an adjacent organ. The regional lymph nodes were considered to be involved by metastases if they measured larger than 8 mm in the short-axis diameter. Tumor progression was evaluated according to the 7th edition of the International Union against Cancer TNM classification.[20,21] The radiologic response of the lymph nodes was evaluated according to version 1.0 of the Response Evaluation Criteria for Solid Tumors.[22] The surgical specimens were pathologically evaluated as grade 0 when degeneration and/or necrosis were absent within the tumor, grade 1a when these areas accounted for less than one-third of the tumor, grade 1b when these areas accounted for more than one-third and less than two-thirds of the tumor, grade 2a when these areas accounted for more

than two-thirds of the tumor, although tumor tissue apparently remained, grade 2b when only minimal tumor cells remained, and grade 3 when no residual tumor was detected.[17] Patients with grade 1b, 2a, 2b, or 3 tumors were classified as responders, whereas those with grade 0 or 1 tumors were classified as nonresponders.

All statistical analyses were performed by using the SPSS version 18.0 software program. Correlations between the two groups were analyzed with the chi-square test.

RESULTS

Between October 2009 and July 2011, a total of 83 patients were enrolled in the COMPASS study. All patients were eligible and received neoadjuvant chemotherapy. Among these 83 patients, 6 did not proceed to surgery because of tumor progression, 2 received bypass surgery because of peritoneal metastasis, and 75 underwent surgical resection and were entered into this study. The background characteristics of these 75 patients are shown in Table 1.

The relationship between the radiologic T and pathologic T stage is demonstrated in Table 2. No significant correlation was found in the 75 patients ($p = 0.221$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 42.7% (32 of 75), 10.7% (8 of 75), and 46.7% (35 of 75), respectively.

A pathologic response of the primary tumor was observed in 32 patients. When stratifying the patients according to the pathologic response (Table 3), the correlation was not significant in either the responders ($n = 32$, $p = 0.410$) or the nonresponders ($n = 43$, $p = 0.742$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 37.5% (12 of 32), 3.1% (1 of 32), and 59.4% (19 of 32), respectively, in the responders and

TABLE 1 Background of the patients ($n = 75$)

Variable	Data	
Age (years)	Median	66
	Range	32–80
Sex	Male/Female	53/22
Performance status	0/1	74/1
Macroscopic type	0	1
	1	5
	2	20
	3	34
	4	8
	5	7
Histologic type	Differentiated	14
	Undifferentiated	56
Clinical T	T2	1
	T3	6
	T4a	64
	T4b	4
Clinical N	N0	12
	N1	37
	N2	17
	N3	9
Regimen	Two courses of S-1 plus cisplatin	20
	Four courses of S-1 plus cisplatin	18
	Two courses of paclitaxel plus cisplatin	18
	Four courses of paclitaxel plus cisplatin	19

TABLE 2 Relationship between clinical T after neoadjuvant chemotherapy and pathologic T

Clinical T	Pathologic T						Total
	T0	T1	T2	T3	T4a	T4b	
T1	0[a]	0[b]	0[c]	0[c]	0[c]	0[c]	0
T2	2[a]	0[a]	2[b]	2[c]	0[c]	0[c]	6
T3	0[a]	3[a]	0[a]	6[b]	4[c]	1[c]	14
T4a	2[a]	3[a]	6[a]	18[a]	24[b]	1[c]	54
T4b	0[a]	0[a]	0[a]	1[a]	0[a]	0[b]	1
Total	4	6	8	27	28	2	75

[a] Overdiagnosis
[b] Accurate diagnosis
[c] Underdiagnosis

TABLE 3 Relationship between clinical T after neoadjuvant chemotherapy and pathologic T by stratifying the pathologic response of the primary tumor

Clinical T	Pathologic T						Total
	T0	T1	T2	T3	T4a	T4b	
Responder							
T1	0[a]	0[b]	0[c]	0[c]	0[c]	0[c]	0
T2	2[a]	0[a]	2[b]	1[c]	0[c]	0[c]	5
T3	0[a]	2[a]	0[a]	4[b]	0[c]	0[c]	6
T4a	2[a]	2[a]	4[a]	5[a]	6[b]	0[c]	19
T4b	0[a]	0[a]	0[a]	0[a]	2[a]	0[b]	2
Total	4	4	6	10	8	0	32
Nonresponder							
T1	0[a]	0[b]	0[c]	0[c]	0[c]	0[c]	0
T2	0[a]	0[a]	0[b]	1[c]	0[c]	0[c]	1
T3	0[a]	1[a]	0[a]	2[b]	4[c]	1[c]	8
T4a	0[a]	1[a]	2[a]	12[a]	18[b]	1[c]	34
T4b	0[a]	0[a]	0[a]	0[a]	0[a]	0[b]	0
Total	0	2	2	15	22	2	43

[a] Overdiagnosis
[b] Accurate diagnosis
[c] Underdiagnosis

46.5% (20 of 43), 16.3% (7 of 43), and 37.2% (16 of 43), respectively, in the nonresponders.

The relationship between the radiologic N and pathologic N stage is shown in Table 4. A significant correlation was found in all 75 patients ($p = 0.000$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 44% (33 of 75), 29.3% (22 of 75), and 26.7% (20 of 75), respectively. For the diagnosis of nodal positivity, the radiologic accuracy, sensitivity, and specificity were 70.7% (53 of 75), 84.9% (45 of 53), and 36.4% (8 of 22), respectively.

Fifty-one patients had measurable lymph nodes according to RECIST version 1.0. Among these patients, a radiologic response was observed in 28 cases. When the 51 patients with measurable lymph nodes were stratified according to the radiologic response (Table 5), the correlation was significant in the nonresponders ($n = 23$, $p = 0.035$) but not in the responders ($n = 28$, $p = 0.634$). The radiologic accuracy and rates of underdiagnosis and overdiagnosis were 39.3% (11 of 28), 21.4% (6 of 28), and 39.3% (11 of 28), respectively, in the responders and 52.1% (12 of 23), 21.7% (5 of 23), and 26.1% (6 of 23), respectively, in the nonresponders.

TABLE 4 Relationship between clinical N after neoadjuvant chemotherapy and pathologic N

Clinical N	Pathologic N				Total
	N0	N1	N2	N3	
N0	8[a]	5[c]	3[c]	0[c]	16
N1	12[b]	9[a]	11[c]	0[c]	32
N2	2[b]	6[b]	15[a]	3[c]	26
N3	0[b]	0[b]	0[b]	1[a]	1
Total	22	20	29	4	75

[a] Accurate diagnosis

[b] Overdiagnosis

[c] Underdiagnosis

TABLE 5 Relationship between clinical N after neoadjuvant chemotherapy and pathologic N by stratifying the radiologic response of the lymph node

Clinical N	Pathologic N				Total
	N0	N1	N2	N3	
Responder					
N0	1[a]	0[c]	1[c]	0[c]	2
N1	6[b]	4[a]	4[c]	0[c]	14
N2	2[b]	3[b]	6[a]	1[c]	12
N3	0[b]	0[b]	0[b]	0[a]	0
Total	9	7	11	1	28
Nonresponder					
N0	0[a]	0[c]	0[c]	0[c]	0
N1	3[b]	3[a]	3[c]	0[c]	9
N2	0[b]	3[b]	8[a]	2[c]	13
N3	0[b]	0[b]	0[b]	1[a]	1
Total	3	6	11	3	23

[a] Accurate diagnosis

[b] Overdiagnosis

[c] Underdiagnosis

Discussion

This study evaluated the accuracy of radiologic diagnosis after neoadjuvant chemotherapy in 75 patients enrolled in the prospective randomized phase II COMPASS study, which predefined radiologic criteria for T- and N-staging. The radiologic overall accuracy was 42.7% for T-staging and 44% for N-staging. Previously, we examined the radiologic accuracy of primary staging determined according to the same criteria using CT in 315 patients with primary resectable gastric cancer and demonstrated that the radiologic accuracy was 71.4% for T-staging and 75.9% for N-staging.[19] Compared with the primary staging, restaging after neoadjuvant chemotherapy was found to be inaccurate and unreliable.

With respect to T-staging after neoadjuvant chemotherapy, the radiologic T and pathologic T stages were not significantly correlated. The overall accuracy was only 42.7%. These results suggest that T-staging using CT provides no clinical information and should not be considered in clinical decision-making. Previously, Park et al.[13] reported that the accuracy of T restaging was 47% on EUS and 57% on CT. The accuracy reported in their study was slightly better than that observed in the present results. In this study, the radiologic accuracy was 37.5% in the responders and 46.5% in the nonresponders, which suggests that the radiologic accuracy is affected by the response of the primary tumor. In Park and colleagues' study, the response rate and accuracy stratified according to the response were not demonstrated.[13]

Most cases of misdiagnosis of the T stage are due to overdiagnosis. Park et al.[13] also reported similar results. Chemotherapy acts on tumor tissue and induces a variety of changes of in both the tumor and stroma, including necrosis, inflammation, and fibrosis.[23] The depth of tumor invasion may become shallow if these changes occur in the tumor tissue. Chemotherapy-induced stromal changes can cause difficulties in distinguishing the wall layer of the stomach on CT, by which overdiagnosis and/or misdiagnosis can occur. When the T stage was examined by separating the patients according to the pathologic response of the primary tumor, the radiologic accuracy was lower and the rate of overdiagnosis was higher in the responders than in the nonresponders. However, the radiologic accuracy was not significantly high, even in the nonresponders. It should be clarified whether chemotherapy-

induced stromal changes occur regardless of the tumor response.

The radiologic N and pathologic N stages were significantly correlated even though the radiologic overall accuracy of N-staging was only 44%. Moreover, the radiologic accuracy and sensitivity of the diagnosis of nodal positivity were both high: 70.7 and 84.9%, respectively. These results suggest that N-staging using CT is not accurate for diagnosing each N category, although it is useful for diagnosing nodal positivity. Previously, Park et al. [13] reported that the accuracy of N restaging was 39% on EUS and 37% on CT, whereas that of nodal positivity was 68% on both EUS and CT in 38 patients. Their results support our data. The sensitivity for diagnosing nodal positivity in this study was high, at 84.9%; however, the specificity was low, at 36.4%, thus suggesting that radiologically determined positive findings are reliable, whereas negative findings are not.

We next examined the accuracy of N-staging by stratifying the radiologic nodal response. The radiologic accuracy was low, at 39.3%, in the responders and higher, at 52.1%, in the nonresponders, which suggests that the radiologic accuracy of N-staging decreases when metastatic nodes respond to chemotherapy. The rates of underdiagnosis and overdiagnosis were almost half in the overall cohort and the nonresponders; however, overdiagnosis was a major cause of misdiagnosis in the responders. Among the responders, eight (89%) of nine patients with pathologic N0 disease were radiologically misdiagnosed as being node-positive. This result suggests that the enlarged nodes did not disappear even though the nodal metastasis pathologically disappeared.

In conclusion, restaging of gastric cancer after neoadjuvant chemotherapy by using CT is inaccurate and unreliable. In particular, the radiologic T stage determined after neoadjuvant chemotherapy should not be considered in clinical decision-making.

ACKNOWLEDGMENT This work was supported by the Epidemiological & Clinical Research Information Network (ECRIN).

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer.* 2010;127:2893–917.
2. Yoshikawa T, Rino Y, Yukawa N, Oshima T, Tsuburaya A, Masuda M. Neoadjuvant chemotherapy for gastric cancer in Japan: a standing position by comparing with adjuvant chemotherapy. *Surg Today.* 2014;44:11–21.
3. Yoshikawa T, Sasako M. Gastrointestinal cancer: adjuvant chemotherapy after D2 gastrectomy for gastric cancer. *Nat Rev Clin Oncol.* 2012;9:192–4.
4. Sakuramoto S, Sasako M, Yamaguchi T, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. *N Engl J Med.* 2007;357:1810–20.
5. Sasako M, Sakuramoto S, Katai H, et al. Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. *J Clin Oncol.* 2011;29:4387–93.
6. Bang YJ, Kim YW, Yang HK, et al. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. *Lancet.* 2012; 379(9813):315–21.
7. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology. Gastric Cancer Version 2.2011. http://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf. Accessed 25 Apr 2013.
8. Allum WH, Blazeby JM, Griffin SM, Cunningham D, Jankowski JA, Wong R. Guidelines for the management of oesophageal and gastric cancer. *Gut.* 2011;60:1449–72.
9. Kwee RM, Kwee TC. Imaging in local staging of gastric cancer: a systematic review. *J Clin Oncol.* 2007;25:2107–16.
10. Kwee RM, Kwee TC. Imaging in assessing lymph node status in gastric cancer. *Gastric Cancer.* 2009;12:6–22.
11. Ajani JA, Mansfield PF, Lynch PM, et al. Enhanced staging and all chemotherapy preoperatively in patients with potentially resectable gastric carcinoma. *J Clin Oncol.* 1999;17:2403–11.
12. Kelsen D, Karpeh M, Schwartz G, et al. Neoadjuvant therapy of high-risk gastric cancer: a phase II trial of preoperative FAMTX and postoperative intraperitoneal fluorouracil-cisplatin plus intravenous fluorouracil. *J Clin Oncol.* 1996;14:1818–28.
13. Park SR, Lee JS, Kim CG, et al. Endoscopic ultrasound and computed tomography in restaging and predicting prognosis after neoadjuvant chemotherapy in patients with locally advanced gastric cancer. *Cancer.* 2008;112:2368–76.
14. Yoshikawa T, Tsuburaya A, Morita S, et al. A comparison of multimodality treatment: two or four courses of paclitaxel plus cisplatin or S-1 plus cisplatin followed by surgery for locally advanced gastric cancer, a randomized phase II trial (COMPASS). *Jpn J Clin Oncol.* 2010;40:369–72.
15. Koizumi W, Narahara H, Hara T, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol.* 2008;9:215–21.
16. Tsuburaya A, Nagata N, Cho H, et al. Phase II trial of paclitaxel and cisplatin as neoadjuvant chemotherapy for locally advanced gastric cancer. *Cancer Chemother Pharmacol.* 2013;71:1309–14.
17. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer.* 2011;14:113–23.
18. Habermann CR, Weiss F, Riecken R, et al. Preoperative staging of gastric adenocarcinoma: comparison of helical CT and endoscopic US. *Radiology.* 2004;230:465–71.
19. Hasegawa S, Yoshikawa T, Shirai J, et al. A prospective validation study to diagnose serosal invasion and nodal metastases of gastric cancer by multidetector-row CT. *Ann Surg Oncol.* 2013;20:2016–22.
20. Sobin LH, Compton CC. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. *Cancer.* 2010;116:5336–9.
21. Sobin LH, Gospodarowicz MK, Witterkind CH. International Union against Cancer (UICC) TNM classification of malignant tumors. 7th ed. Oxford, UK: Wiley-Blackwell; 2009.
22. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst.* 2000;92:205–16.
23. Yonemura Y, Kinoshita K, Fujimura T, et al. Correlation of the histological effects and survival after neoadjuvant chemotherapy on gastric cancer patients. *Hepatogastroenterology.* 1996;43: 1260–72.

Mol Diagn Ther
DOI 10.1007/s40291-014-0103-6

ORIGINAL RESEARCH ARTICLE

FcγR and *EGFR* Polymorphisms as Predictive Markers of Cetuximab Efficacy in Metastatic Colorectal Cancer

Yuka Inoue · Shoichi Hazama · Shigeyoshi Iwamoto · Yasuhiro Miyake · Chu Matsuda · Ryouichi Tsunedomi · Naoko Okayama · Yuji Hinoda · Takahiro Yamasaki · Yutaka Suehiro · Shigefumi Yoshino · Junichi Sakamoto · Hideyuki Mishima · Masaaki Oka

Abstract

Background and Objectives Cetuximab shows activity in *KRAS* (Kirsten rat sarcoma viral oncogene homolog) wild-type metastatic colorectal cancer (mCRC). Recent studies have demonstrated that cetuximab induces antibody-dependent cell-mediated cytotoxicity (ADCC) in mCRC. We investigated the associations of *FcγR* (fragment C γ receptor) and *EGFR* (epidermal growth factor receptor) polymorphisms with the outcome of mCRC patients treated with cetuximab and FOLFIRI (folic acid/5-fluorouracil/irinotecan) as second-line therapy in the FLIER (Cetuximab Plus Folinic Acid/5-Fluorouracil/Irinotecan in *KRAS* Wild-Type Metastatic Colorectal Cancer as a Second-Line Treatment) study.

Methods A total of 57 patients were evaluated in this study. The association of each polymorphism with the response rate, progression-free survival, and overall survival was analyzed.

Results A tendency for longer overall survival was observed in patients with the *EGFR* CA repeat ≥36 genotype than in those with the ≤35 genotype (600 versus 483 days, $P = 0.051$). The haplotype containing the 131H and 158V alleles was associated with a lower response rate than the other haplotypes ($P = 0.018$). These results are contrary to previously published results.

Conclusion Our data suggest that *FcγR* and *EGFR* CA repeat polymorphisms may be associated with the outcome of mCRC patients treated with cetuximab and FOLFIRI, although further investigations will be needed to confirm the association of *FcγR* and *EGFR* polymorphisms with the efficacy of cetuximab.

Electronic supplementary material The online version of this article (doi:10.1007/s40291-014-0103-6) contains supplementary material, which is available to authorized users.

Y. Inoue · S. Hazama (✉) · R. Tsunedomi · S. Yoshino · M. Oka
Department of Digestive Surgery and Surgical Oncology (Surgery II), Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
e-mail: hazama@yamaguchi-u.ac.jp

S. Iwamoto
Kansai Medical University Hirakata Hospital, Hirakata 573-1191, Japan

Y. Miyake
Nishinomiya Municipal Central Hospital, Nishinomiya 663-8014, Japan

C. Matsuda
Osaka General Medical Center, Osaka 558-8558, Japan

N. Okayama · Y. Hinoda · T. Yamasaki · Y. Suehiro
Department of Oncology and Laboratory Medicine, Yamaguchi University Graduate School of Medicine, Ube 755-8505, Japan

J. Sakamoto
Tokai Central Hospital, Kakamigahara 504-8601, Japan

H. Mishima
Cancer Center, Aichi Medical University, Nagakute 480-1195, Japan

Published online: 15 May 2014

Key Points

A multivariate analysis has shown that *EGFR* (epidermal growth factor receptor) CA repeat polymorphism was the only independent predictor of overall survival.

A tendency for longer overall survival was observed in patients with the *EGFR* CA repeat ≥36 genotype than in those with the ≤35 genotype.

The haplotype containing the 131H and 158V alleles was significantly associated with a lower response rate than the other haplotypes.

1 Introduction

Colorectal cancer has a high incidence and high mortality worldwide. In 2012, colorectal cancer was the second most prevalent cancer among males (9 %) and the third among females (8 %) [1]. Approximately 92,000 new cases of colorectal cancer are diagnosed each year in Japan [2]. Cetuximab, a chimeric immunoglobulin G1 (IgG1) monoclonal antibody targeted against the extracellular domain of *EGFR* (epidermal growth factor receptor) has shown efficacy in patients with metastatic colorectal cancer (mCRC) [3, 4]. Since it was established that *KRAS* (Kirsten rat sarcoma viral oncogene homolog) mutations are a major negative predictor of efficacy [5, 6], additional biomarkers have emerged in attempts to improve the selection of patients who are likely to be responsive to cetuximab.

Modulation of the immune response could be another important mechanism of cetuximab sensitivity. Antibody-dependent cellular cytotoxicity mediated through the fragment C γ receptor (FcγR) carried by immune cells, such as macrophages and natural killer cells, plays an important role in the antitumor effect of the IgG1 antibody [7]. Constitutional polymorphisms on genes encoding the activating receptors FcγRIIa (Fc fragment of IgG, low affinity IIa, receptor), which is mainly expressed on macrophages, and FcγRIIIa (Fc fragment of IgG, low affinity IIIa, receptor), which is expressed on natural killer cells and macrophages, affect their affinity for human IgG. These polymorphisms consist of a histidine (H)/arginine (R) polymorphism at position 131 on *FcγRIIa* and a valine (V)/phenylalanine (F) polymorphism at position 158 on *FcγRIIIa* [8]. On the basis of the different binding affinities, patients harboring *FcγRIIa*-131H/H and *FcγRIIIa*-158V/V genotypes would be expected to mediate a more potent antibody-dependent cell-mediated cytotoxicity (ADCC) antitumor response after monoclonal antibody treatment [9–11]. Clinical studies have shown that the *FcγRIIa*-131H/H [12, 13] and *FcγRIIIa*-158V/V genotypes [12–14] are associated with better clinical outcomes of rituximab as first-line treatment for follicular lymphoma and trastuzumab-based therapy in metastatic breast cancer. In vitro studies have shown that cetuximab is able to induce ADCC [15, 16]. However, conflicting data have been published regarding the allele best able to predict the clinical response of mCRC patients treated with cetuximab [17–19].

Additionally, the number of CA single sequence repeats in intron 1 of the *EGFR* gene, which affects the transcription efficiency of the gene, is associated with the response to EGFR antibodies. Several studies have suggested a correlation between polymorphic variations in intron 1 of the *EGFR* gene and the response to EGFR inhibitors. Head and neck cancer cells with a smaller number of *EGFR* intron 1 CA repeats had higher EGFR expression and greater sensitivity to anti-EGFR therapy [20], and mCRC patients with a smaller number of *EGFR* intron 1 CA repeats showed greater sensitivity to treatment with cetuximab and irinotecan [21].

A polymorphic variant in *EGFR* arising from a single nucleotide substitution (142285 G>A) leads to an arginine (R) to lysine (K) substitution in codon 521 of the extracellular domain of *EGFR* [22]. Compared with the wild-type 521R allele, the 521K allele variant has attenuated affinity in ligand binding and proto-oncogene induction, which may affect the efficacy of cetuximab [22–24].

The aim of the present study was to evaluate the influence of *FcγRIIa*, *FcγRIIIa*, and *EGFR* polymorphisms and the number of CA single sequence repeats in intron 1 of the *EGFR* gene on the outcome of 57 patients with oxaliplatin-resistant mCRC treated with cetuximab plus FOLFIRI (folic acid/5-fluorouracil/irinotecan).

2 Patients and Methods

2.1 Eligible Patients

We conducted a study of cetuximab plus FOLFIRI in advanced/metastatic colorectal cancer as second-line therapy for *KRAS* codon 12/13 wild-type colorectal cancer. This study was called the FLIER (Cetuximab Plus Folinic Acid/5-Fluorouracil/Irinotecan in *KRAS* Wild-Type Metastatic Colorectal Cancer as a Second-Line Treatment) study (UMIN000002094) and was a multicenter phase II study. The eligibility criteria were as follows. The patients included in the study had histologically proven, unresectable mCRC with at least one measurable lesion, according to the Response Evaluation Criteria in Solid Tumors (RECIST). They had an Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ

function for the study treatment. All patients were at least 20 years old and had a life expectancy of at least 3 months. They had previously received at least one regimen of oxaliplatin-containing chemotherapy at least 28 days prior to the first study treatment. For inclusion in the study, each patient's primary or metastatic tumor tissue needed to have immunohistochemically confirmed EGFR expression and *KRAS* wild-type sequences at codons 12 and 13. Each of the patients included in the study had previously had surgery, and samples of their tumors had been stored [25]. In our analysis, the patients with *BRAF* (v-raf murine sarcoma viral oncogene homolog B) mutations were excluded because of their extremely poor prognosis. The study protocol was performed according to the Declaration of Helsinki and was approved by the institutional review board at Yamaguchi University, as well as at each study site. All patients provided written informed consent before initiation of study-related procedures. All patients were treated with cetuximab at a standard loading dose of 400 mg/m^2 over a 2 h period, followed by a weekly infusion of 250 mg/m^2 over a 1 h period, combined with the standard dosage for FOLFIRI in Japan. Briefly, FOLFIRI consisted of irinotecan 150 mg/m^2 plus leucovorin 200 mg/m^2 administered intravenously for 90 min, followed by a fluorouracil 400 mg/m^2 bolus on day 1 and a fluorouracil 2,400 mg/m^2 continuous infusion for 46 h every 2 weeks.

2.2 Clinical Evaluation and Response Criteria

For all patients with measurable disease, the response was evaluated every 2 months during the course of the study by computed tomography, according to RECIST version 1.0, and classified as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).

2.3 Immunohistochemical Analysis of EGFR Expression

Paraffin-embedded tissues fixed in 10 % neutral buffered formalin were cut at 4 μm thickness. Immunostaining of the sections was performed using the EGFR pharmDx™ kit (Dako, Glostrup, Denmark), in accordance with the manufacturer's instructions. EGFR expression was defined as membranous immunohistological brown staining of tumor cells. Tumor cells with any membrane staining above the background level, whether this was complete or incomplete circumferential staining, were considered positive for EGFR expression. The primary tumor was considered positive when 1 % of tumor cells had membranous staining.

2.4 Examination of *KRAS* and *BRAF* Mutations and *EGFR* CA Repeats in Intron 1 Genotyping

DNA extraction was performed using a QIAamp DNA FFPE Tissue kit (Qiagen, Tokyo, Japan), in accordance with the manufacturer's instructions. Mutation of *KRAS* at codons 12 and 13 and *BRAF* at codon 600 were determined by direct sequencing, as previously described [26, 27].

2.5 Examination of *FcγRIIa*, *FcγRIIIa*, and *EGFR* Genotyping

Peripheral blood was treated with ethylenediaminetetraacetic acid, disodium salt (EDTA-2Na) as an anticoagulant, and genomic DNA was extracted using a conventional NaI method [28]. *FcγRIIa*-H131R rs180127, *FcγRIIIa*-V158F rs396991, and *EGFR*-R521K rs2227983 polymorphisms were genotyped using the TaqMan technique. The primer sets used for amplifications were the TaqMan SNP Genotyping Assays C 9077561 10, C 25815666 10, and C 16170352 20 (Applied Biosystems, Foster City, CA, USA). The reactions were performed in 384-well microtiter plates. The assay volume was 5 μL and contained TaqMan Genotyping Master Mix (Applied Biosystems), Assay Mix, and 20–40 ng of genomic DNA diluted in dH_2O. The PCR profile was 2 min at 50 °C (to degrade dU-containing DNA), 10 min at 95 °C (denaturation), and 40 cycles of 15 s at 95 °C and 1 min of annealing and extension at 60 °C. End-point reading of the fluorescence generated during the PCR amplification was performed using an ABI Prism 7900HT (Applied Biosystems).

2.6 Statistical Analysis

Fisher's exact test and trend test were used to calculate *P* values for the associations between genetic parameters and response rates.

OS was defined as the time from registration to death from any cause. Progression-free survival (PFS) was also analyzed, and it was defined as the time from registration to the first appearance of progression or death from any cause.

The association between the patient characteristics and OS was examined using Cox proportional hazards regression models; we report hazard ratio (HR) estimates and their 95 % confidence intervals (CIs). The association of each polymorphism with OS and PFS was analyzed using Kaplan–Meier curves and the log-rank test. All statistical analyses were performed using SPSS for Windows version 20 software (SPSS, Chicago, IL, USA). All *P* values were two-sided and considered statistically significant when <0.05.

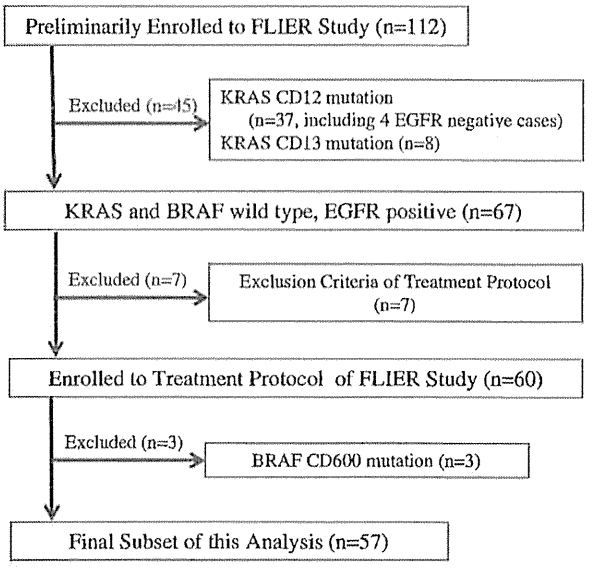

Fig. 1 Consolidated Standards of Reporting Trials (CONSORT) diagram for the FLIER (Cetuximab Plus Folinic Acid/5-Fluorouracil/Irinotecan in *KRAS* Wild-Type Metastatic Colorectal Cancer as a Second-Line Treatment) study. *BRAF* v-raf murine sarcoma viral oncogene homolog B, *CD* codon, *EGFR* epidermal growth factor receptor, *KRAS* Kirsten rat sarcoma viral oncogene homolog

3 Results

Between December 2008 and December 2009, 112 patients were preliminarily enrolled; of these 112 patients, 37 with a *KRAS* codon (CD) 12 mutation, including four EGFR-negative cases, and eight patients with a *KRAS* CD13 mutation were excluded. Of 67 patients with *KRAS* exon2 wild-type and EGFR-positive tumors, 57 eligible patients were finally enrolled in the FLIER study. Three patients with a *BRAF* CD600 mutation were excluded, and 57 patients formed the final subset for this analysis. The Consolidated Standards of Reporting Trials (CONSORT) diagram is shown in Fig. 1. The characteristics of the 57 patients are summarized in Table 1.

Table 1 Patient characteristics ($N = 57$)

Characteristic	Value
Age (years)	
Average	62.7
Range	37–82
Sex (n)	
Male	37
Female	20
Pattern of metastatic disease (n)	
Liver	35
Lung	21
Other	17
First-line regimen (n)	
FOLFOX + bevacizumab	29
FOLFOX	14
FOLFOX + cediranib/placebo	11
Other	3

FOLFOX bolus/infusional fluorouracil and leucovorin with oxaliplatin

3.1 Association of Overall Survival with Patient Characteristics

Associations between the patient characteristics and OS are summarized in Table 2. A multivariate analysis showed that *EGFR* CA repeats were the only independent predictor of OS (HR 1.97, 95 % CI 1.02–3.78, $P = 0.04$).

3.2 Association of Objective Response with Each Polymorphism

Associations between each genotype and the objective response are summarized in Table 3. The *FcγRIIa*, *FcγRIIIa*, *EGFR* (R521K), and *EGFR* CA repeat genotypes were not significantly associated with the response rate ($P = 0.487$, $P = 0.410$, $P = 0.934$, $P = 0.220$, respectively; Fisher's exact test). The result of LD analysis is shown in Supporting Figure S1.

3.3 Association of Progression-Free Survival and Overall Survival with Each Polymorphism

The associations of each genotype with PFS and OS are summarized in Table 4. A tendency for longer OS was observed in patients with the *EGFR* CA repeat $\geq$36 genotype than in those with the $\leq$35 genotype (600 versus 483 days, $P = 0.051$; log-rank test). There was no association of *FcγRIIa*, *FcγRIIIa*, *EGFR* (R521K), and *EGFR* CA repeat genotypes with PFS.

3.4 Association of *FcγR* Haplotype and Diplotype with Response

The association between *FcγR* haplotypes and response are summarized in Table 5. The haplotype containing the 131H and 158V alleles was significantly associated with a lower response rate than the other haplotypes ($P = 0.018$; Fisher's exact test). There was no association of *FcγR* haplotypes with PFS or OS (data not shown). The associations between *FcγR* diplotypes and response are summarized in Table 6. The diplotypes containing 131H and 158V alleles had a significantly lower response rate than the other diplotypes ($P = 0.038$; trend test). There was no association of *FcγR* diplotypes with PFS or OS (data not shown).

Table 2 Associations of patient characteristics with overall survival

Characteristic	*n*	Univariate analysis			Multivariate analysis		
		HR	95 % CI	*P* value	HR	95 % CI	*P* value
Age							
≤65 years	35	0.95	0.53–1.70	0.86	0.84	0.46–1.53	0.57
≥65 years	22						
Sex							
Male	37	1.12	0.62–2.04	0.70	1.22	0.66–2.23	0.53
Female	20						
Number of metastatic sites							
1	12	1.20	0.60–2.41	0.61			
≥2	45						
EGFR (CA repeats)							
≤35	15	1.86	0.99–3.52	0.06	1.97	1.02–3.78	0.04
≥36	42						
FcγR polymorphisms							
Both FcγRIIa-131H and FcγRIIIa-158V	17	0.94	0.50–1.76	0.84			
Other	40						

CI confidence interval, *EGFR* epidermal growth factor receptor, *FcγR* fragment C γ receptor, *HR* hazard ratio

Table 3 Associations of *FcγR* (fragment C γ receptor) and *EGFR* (epidermal growth factor receptor) genotypes with objective response

Genotype	Detail	Objective response			
		Response (*n*)		*P* value	
		CR + PR	SD + PD	Fisher's exact test	C.–A. trend test
FcγRIIa (H131R)	HH	10	25	0.487	0.239
	HR	6	9		
	RR	3	3		
FcγRIIIa (V158F)	FF	15	22	0.410	0.173
	FV	3	11		
	VV	1	4		
EGFR (R521K)	RR	7	12	0.934	0.613
	KR	9	17		
	KK	3	8		
EGFR (CA repeats)	≤35	3	12	0.220	
	≥36	16	25		

C.–A. Cochran–Armitage, *CR* complete response, *PD* progressive disease, *PR* partial response, *SD* stable disease

4 Discussion

Two important findings were obtained from the present study. First, the haplotype containing the *FcγRIIa*-131H and *FcγRIIIa*-158V alleles was significantly associated with a lower response rate than the other haplotypes (Table 5; $P = 0.018$; Fisher's exact test), and the diplotypes containing the *FcγRIIa*-131H and *FcγRIIIa*-158V alleles also had significantly lower response rates than the other diplotypes (Table 6; $P = 0.038$; trend test). Several studies have attempted to correlate clinical outcomes with *FcγR* polymorphisms that are known to modulate antibody–receptor engagement, but the results were not consistent. *FcγRIIIa*-158F/F has previously been correlated with a higher response rate and with longer PFS in mCRC patients treated with cetuximab [18, 19], and those findings are supportive of our results. In contrast, some reports have demonstrated that the *FcγRIIIa*-158V/V genotype confers higher binding affinity for IgG1 in vitro [10], and patients harboring this genotype have greater responsiveness to herceptin and rituximab [13, 14]. In another trial of mCRC treated with cetuximab, improved clinical outcomes in *FcγRIIIa*-158V/V carriers were reported [17]. Combined analysis of *FcγRIIa* and *FcγRIIIa* polymorphisms was performed in several studies. Patients with any *FcγRIIa*-131H allele and/or *FcγRIIIa*-158F allele had a higher response rate and longer PFS [18], whereas patients with any *FcγRIIa*-131H and/or *FcγRIIIa*-158V allele had a

Table 4 Associations of *FcγR* (fragment C γ receptor) and *EGFR* (epidermal growth factor receptor) polymorphisms with overall survival and progression-free survival

Polymorphism	Detail	*n*	Progression-free survival		Overall survival	
			Median (days)	*P* value of log-rank test	Median (days)	*P* value of log-rank test
FcγRIIa (H131R)	HH	35	254	0.747	553	0.991
	HR	16	203		663	
	RR	6	277		549	
FcγRIIIa (V158F)	FF	37	277	0.907	553	0.234
	FV	15	286		737	
	VV	5	154		270	
EGFR (R521K)	RR	11	230	0.236	566	0.386
	KR	26	315		553	
	KK	20	159		330	
EGFR (CA repeats) (≤35, ≥36)	S	15	307	0.954	483	0.051
	L	42	254		600	

L ≥36 CA repeats, *OS* overall survival, *PFS* progression-free survival, *S* ≤35 CA repeats

Table 5 Associations of *FcγR* (fragment C γ receptor) haplotypes with objective response

Haplotype of *FcγRIIa*-H131R and *FcγRIIIa*-V158F	Allele frequency	Objective response ($N = 112$)		
		Response (*n*)		*P* value
		CR + PR	SD + PD	Fisher's exact test
HF	0.589	24	42	0.549
Without HF		14	32	
RF	0.196	9	13	0.460
Without RF		29	61	
HV	0.170	2	17	0.018
Without HV		36	57	
RV	0.045	3	2	0.334
Without RV		35	72	

CR complete response, *PD* progressive disease, *PR* partial response, *SD* stable disease

Table 6 Associations of *FcγR* (fragment C γ receptor) diplotypes with objective response

Diplotype of FcγRIIa-H131R and FcγRIIIa-V158F	Allele frequency	Objective response ($N = 56$)			
		Response [*n*]		*P* value	
		CR + PR	SD + PD	Fisher's exact test	C.–A. trend test
HV/HV	0.071	0	4	0.162	0.038
HV/non-HV	0.196	2	9		
Non-HV/non-HV	0.732	17	24		

C.–A. Cochran–Armitage, *CR* complete response, *PD* progressive disease, *PR* partial response, *SD* stable disease

higher disease control rate [29] and longer PFS [17]. These discrepancies might be explained as follows. Although ADCC induction is clear in in vitro models, some problems have been raised in cancer patients. ADCC is markedly impaired in cancer patients, especially patients with advanced disease and a high tumor burden, because of frequently observed natural killer cell dysfunction [30]. In addition, the tumor microenvironment enhances the expansion of immunosuppressive cells, such as regulatory T cells and myeloid-derived suppressor cells [31], which impair the ADCC activity induced by cetuximab.

Second, our study demonstrated that patients with the *EGFR* genotype with 36 or more CA single sequence repeats in intron 1 had a tendency for longer OS than

patients with 35 or fewer CA repeats (600 versus 483 days, $P = 0.051$; log-rank test; Table 3). The number of CA repeats in the *EGFR* gene affects the transcription efficiency of the gene, with transcription levels declining with increasing numbers of CA repeats in vitro [32], and is also associated with the response to EGFR antibodies. Head and neck cells with a smaller number of *EGFR* intron 1 CA repeats have shown higher EGFR expression and greater sensitivity to anti-EGFR therapy in vitro [20], and mCRC patients with a smaller number of *EGFR* intron 1 CA repeats have shown greater sensitivity to cetuximab and irinotecan than patients with a larger number of CA repeats [19, 21]. The data from the previous studies conflict with our data.

Furthermore, our study found no association of *EGFR* R521K polymorphism with the response rate, PFS, and OS. Compared with the wild-type 521R allele, the 521K allele variant has attenuated affinity in ligand binding and proto-oncogene induction, which may affect the efficacy of cetuximab [22–24]. This polymorphism has served as a predictive marker for cetuximab-based treatment in *KRAS* wild-type colorectal cancer, as patients with the KR or KK genotypes have been found to have a significantly higher response rate and longer PFS and OS than patients with the RR genotype [24].

These discrepancies regarding the association between *EGFR* polymorphisms and the outcome of cetuximab treatment might be explained as follows. A second subgroup analysis of the BOND study showed that the degree of EGFR expression—either the percentage of EGFR-expressing tumor cells or the maximal staining intensity per cell—did not correlate significantly with the clinical response rate [33]. This finding could be the explanation for the conflicting results.

5 Conclusion

Our data suggest that *FcγR* and *EGFR* CA repeat polymorphisms may be associated with the outcome of *KRAS*-wild type mCRC in patients treated with cetuximab and FOLFIRI as second-line therapy, although these results are controversial now, and further investigations will be needed to confirm the association of *FcγR* and *EGFR* polymorphisms with the efficacy of cetuximab.

Acknowledgments This study was supported in part by a non-profit organization, the Epidemiological and Clinical Research Information Network (ECRIN), and by Japan Society for the Promotion of Science (JSPS) KAKENHI grant numbers 21591725 and 19591545. We thank Ms. Mai Hatta for her excellent clinical research coordination.

Disclosure Statement The authors have no conflicts of interest that are directly relevant to the content of this article.

References

1. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012;62(4):220–41. doi:10.3322/caac.21149.
2. Tsukuma H, Ajiki W, Ioka A, Oshima A. Survival of cancer patients diagnosed between 1993 and 1996: a collaborative study of population-based cancer registries in Japan. Jpn J Clin Oncol. 2006;36(9):602–7. doi:10.1093/jjco/hyl068.
3. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, et al. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet. 2011;377(9783):2103–14. doi:10.1016/s0140-6736(11)60613-2.
4. Tveit KM, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. J Clin Oncol. 2012;30(15):1755–62. doi:10.1200/jco.2011.38.0915.
5. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66(8):3992–5. doi:10.1158/0008-5472.can-06-0191.
6. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359(17):1757–65. doi:10.1056/NEJMoa0804385.
7. Weiner GJ. Monoclonal antibody mechanisms of action in cancer. Immunol Res. 2007;39(1–3):271–8.
8. van Sorge NM, van der Pol WL, van de Winkel JG. FcgammaR polymorphisms: implications for function, disease susceptibility and immunotherapy. Tissue Antigens. 2003;61(3):189–202.
9. Parren PW, Warmerdam PA, Boeije LC, Arts J, Westerdaal NA, Vlug A, et al. On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest. 1992;90(4):1537–46. doi:10.1172/jci116022.
10. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood. 1997;90(3):1109–14.
11. Strome SE, Sausville EA, Mann D. A mechanistic perspective of monoclonal antibodies in cancer therapy beyond target-related effects. Oncologist. 2007;12(9):1084–95. doi:10.1634/theoncologist.12-9-1084.
12. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol. 2003;21(21):3940–7. doi:10.1200/jco.2003.05.013.
13. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol. 2008;26(11):1789–96. doi:10.1200/jco.2007.14.8957.
14. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood. 2002;99(3):754–8.
15. Kawaguchi Y, Kono K, Mimura K, Sugai H, Akaike H, Fujii H. Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma. Int J Cancer. 2007;120(4):781–7. doi:10.1002/ijc.22370.

16. Kurai J, Chikumi H, Hashimoto K, Yamaguchi K, Yamasaki A, Sako T, et al. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. Clin Cancer Res. 2007;13(5):1552–61. doi:10.1158/1078-0432.ccr-06-1726.
17. Bibeau F, Lopez-Crapez E, Di Fiore F, Thezenas S, Ychou M, Blanchard F, et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol. 2009;27(7):1122–9. doi:10.1200/jco.2008.18.0463.
18. Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. J Clin Oncol. 2007;25(24):3712–8. doi:10.1200/jco.2006.08.8021.
19. Pander J, Gelderblom H, Antonini NF, Tol J, van Krieken JH, van der Straaten T, et al. Correlation of FCGR3A and EGFR germline polymorphisms with the efficacy of cetuximab in KRAS wild-type metastatic colorectal cancer. Eur J Cancer. 2010; 46(10):1829–34. doi:10.1016/j.ejca.2010.03.017.
20. Amador ML, Oppenheimer D, Perea S, Maitra A, Cusatis G, Iacobuzio-Donahue C, et al. An epidermal growth factor receptor intron 1 polymorphism mediates response to epidermal growth factor receptor inhibitors. Cancer Res. 2004;64(24):9139–43. doi:10.1158/0008-5472.can-04-1036.
21. Graziano F, Ruzzo A, Loupakis F, Canestrari E, Santini D, Catalano V, et al. Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. J Clin Oncol. 2008;26(9):1427–34. doi:10.1200/jco.2007.12.4602.
22. Zhang W, Stoehlmacher J, Park DJ, Yang D, Borchard E, Gil J, et al. Gene polymorphisms of epidermal growth factor receptor and its downstream effector, interleukin-8, predict oxaliplatin efficacy in patients with advanced colorectal cancer. Clin Colorectal Cancer. 2005;5(2):124–31.
23. Goncalves A, Esteyries S, Taylor-Smedra B, Lagarde A, Ayadi M, Monges G, et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. BMC Cancer. 2008;8:169. doi:10.1186/1471-2407-8-169.
24. Hsieh YY, Tzeng CH, Chen MH, Chen PM, Wang WS. Epidermal growth factor receptor R521K polymorphism shows favorable outcomes in KRAS wild-type colorectal cancer patients treated with cetuximab-based chemotherapy. Cancer Sci. 2012;103(4):791–6. doi:10.1111/j.1349-7006.2012.02225.x.
25. Iwamoto S, Hazama S, Kato T, Miyake Y, Fukunaga M, Matsuda C, et al. Multicenter phase II study of second-line cetuximab plus folinic acid/5-fluorouracil/irinotecan (FOLFIRI) in KRAS wild-type metastatic colorectal cancer: the FLIER study. Anticancer Res. 2014;34(4):1967–73.
26. Okayama N, Nishioka M, Hazama S, Sakai K, Suehiro Y, Maekawa M, et al. The importance of evaluation of DNA amplificability in KRAS mutation testing with dideoxy sequencing using formalin-fixed and paraffin-embedded colorectal cancer tissues. Jpn J Clin Oncol. 2011;41(2):165–71. doi:10.1093/jjco/hyq173.
27. Maruta Y, Okayama N, Hiura M, Suehiro Y, Hirai H, Hinoda Y. Determination of ancestral allele for possible human cancer-associated polymorphisms. Cancer Genet Cytogenet. 2008; 180(1):24–9. doi:10.1016/j.cancergencyto.2007.09.011.
28. Wang L, Hirayasu K, Ishizawa M, Kobayashi Y. Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS. Nucleic Acids Res. 1994;22(9):1774–5.
29. Rodriguez J, Zarate R, Bandres E, Boni V, Hernandez A, Sola JJ, et al. Fc gamma receptor polymorphisms as predictive markers of cetuximab efficacy in epidermal growth factor receptor downstream-mutated metastatic colorectal cancer. Eur J Cancer. 2012;48(12):1774–80. doi:10.1016/j.ejca.2012.01.007.
30. Kono K, Takahashi A, Ichihara F, Sugai H, Fujii H, Matsumoto Y. Impaired antibody-dependent cellular cytotoxicity mediated by herceptin in patients with gastric cancer. Cancer Res. 2002; 62(20):5813–7.
31. Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A. CD4+ CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. Cancer. 2003;98(5):1089–99. doi:10.1002/cncr.11618.
32. Gebhardt F, Zanker KS, Brandt B. Modulation of epidermal growth factor receptor gene transcription by a polymorphic dinucleotide repeat in intron 1. J Biol Chem. 1999;274(19):13176–80.
33. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004;351(4):337–45. doi:10.1056/NEJMoa033025.

Ann Surg Oncol
DOI 10.1245/s10434-014-3670-1

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – GASTROINTESTINAL ONCOLOGY

Low Creatinine Clearance is a Risk Factor for D2 Gastrectomy after Neoadjuvant Chemotherapy

Tsutomu Hayashi, MD[1], Toru Aoyama, MD[1], Kazuaki Tanabe, MD[2], Kazuhiro Nishikawa, MD[3], Yuichi Ito, MD[4], Takashi Ogata, MD[1], Haruhiko Cho, MD[1], Satoshi Morita, PhD[5], Yumi Miyashita[6], Akira Tsuburaya, MD[7], Junichi Sakamoto, MD[8], and Takaki Yoshikawa, MD[1]

[1]Department of Gastrointestinal Surgery, Kanagawa Cancer Center, Asahi-Ku, Yokohama, Japan; [2]Department of Gastrointestinal Surgery, Hiroshima University, Hiroshima, Japan; [3]Department of Surgery, Osaka General Medical Center, Osaka, Japan; [4]Department of Gastroenterological Surgery, Aichi Cancer Center, Nagoya, Japan; [5]Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, Yokohama, Japan; [6]Data center, Nonprofit Organization ECRIN, Okazaki, Japan; [7]Clinical Research Center, Shonan Kamakura General Hospital, Kamakura, Japan; [8]Tokai Central Hospital, Kakamigahara, Japan

ABSTRACT

Background. The feasibility and safety of D2 surgery following neoadjuvant chemotherapy (NAC) has not been fully evaluated in patients with gastric cancer. Moreover, risk factor for surgical complications after D2 gastrectomy following NAC is also unknown. The purpose of the present study was to identify risk factors of postoperative complications after D2 surgery following NAC.

Methods. This study was conducted as an exploratory analysis of a prospective, randomized Phase II trial of NAC. The surgical complications were assessed and classified according to the Clavien–Dindo classification. A uni- and multivariate logistic regression analyses were performed to identify risk factors for morbidity.

Results. Among 83 patients who were registered to the Phase II trial, 69 patients received the NAC and D2 gastrectomy. Postoperative complications were identified in 18 patients and the overall morbidity rate was 26.1 %. The results of univariate and multivariate analyses of various factors for overall operative morbidity, creatinine clearance (CCr) $\leq$ 60 ml/min ($P = 0.016$) was identified as sole significant independent risk factor for overall morbidity. Occurrence of pancreatic fistula was significantly higher in the patients with a low CCr than in those with a high CCr.

Conclusions. Low CCr was a significant risk factor for surgical complications in D2 gastrectomy after NAC. Careful attention is required for these patients.

Gastric cancer is the second most frequent cancer-related cause of death after lung cancer.[1] Complete resection is essential for the cure of gastric cancer. After the long debate, D2 gastrectomy has been established as a standard surgical procedure not only in Japan and Korea, but also in Europe and United States.[2–5]

Recently, several large phase III studies demonstrated that multimodality treatment, including surgery, significantly improved the survival of locally advanced disease compared with surgery alone; postoperative adjuvant chemotherapy with S-1 in Japan, postoperative adjuvant chemotherapy with capecitabine plus oxaliplatin in Korea, and pre- and postoperative chemotherapy in United Kingdom.[6–8] Neoadjuvant chemotherapy is a promising treatment for gastric cancer when considering intensive chemotherapy with relatively toxic regimen.[9] Neoadjuvant chemotherapy is tested in several phase III trials in the eastern Asia where D2 and adjuvant chemotherapy is a standard treatment.[10,11] Although D2 gastrectomy is a feasible and safe procedure as a primary treatment when performed by experienced surgeons, the feasibility and safety of D2 surgery following neoadjuvant chemotherapy has not been fully evaluated. Two positive phase III trials performed in United Kingdom and France demonstrated

Tsutomu Hayashi and Toru Aoyama have contributed equally to this work.

First Received: 12 July 2013

T. Yoshikawa, MD
e-mail: yoshikawat@kcch.jp

Published online: 09 April 2014