Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Impact of Expression of Human Epidermal Growth Factor Receptors EGFR and ERBB2 on Survival in Stage II/III Gastric Cancer

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Abstract

Purpose: EGF receptor (EGFR) and HER2 positivity are considered to be negative prognostic factors in gastric cancer. Biomarker analysis was conducted to evaluate the impact of EGFR and HER2 expression on the outcome of patients enrolled in the Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC), a randomized controlled trial comparing postoperative adjuvant S-1 therapy with surgery alone in 1,059 patients with stage II/III gastric cancer.

Experimental Design: Formalin-fixed, paraffin-embedded surgical specimens were retrospectively examined in 829 patients (78.3%). The effects of EGFR and HER2 positivity on survival were analyzed on the basis of the 5-year survival data from the study. EGFR positivity was defined as an immunohistochemistry (IHC) score of 3+, and HER2 positivity as an IHC score of 3+ or an IHC score of 2+ with a positive dual-color *in situ* hybridization status.

Results: EGFR and HER2 were positive in 75 (9.0%) and 113 (13.6%) patients, respectively. The overall and relapse-free survival rates were significantly lower in EGFR-positive patients than in EGFR-negative patients, whereas they were similar in HER2-positive and HER2-negative patients. Multivariate analysis showed that EGFR positivity correlated with poor outcomes [HR = 1.504; 95% confidence interval (CI) = 1.020–2.149; $P = 0.040$]. Treatment with S-1 improved survival compared with surgery alone, irrespective of EGFR and HER2 status.

Conclusions: EGFR positivity, but not HER2 positivity, was associated with poor patient outcomes after curative resection of stage II/III gastric cancer. There was no interaction between S-1 and EGFR or HER2 status with respect to survival outcome. *Clin Cancer Res; 18(21); 5992–6000. ©2012 AACR.*

Introduction

Gastric cancer is the second leading cause of cancer-related deaths worldwide, and the highest mortality rates have been reported in East Asia, including Japan, Korea, and China (28.1 per 100,000 males, 13.0 per 100,000 females; ref. 1). The mainstay of treatment of gastric cancer is surgery; however, in stage II (excluding T1 disease) and stage III (moderately advanced) disease, many patients suffer recurrence, even after curative resection. Various regimens for adjuvant chemotherapy have been implemented to prevent this.

S-1 (TS-1; Taiho Pharmaceutical Co. Ltd.) is an oral fluoropyrimidine preparation, combining tegafur, gimeracil, and oteracil potassium (2). The Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC), which was a prospective randomized phase III trial, showed that S-1 was more effective than surgery alone in East Asian patients with stage II/III gastric cancer (3, 4). However, the 5-year overall survival (OS) rate in patients with stage IIIB disease was 50.2% in the S-1 group in a subset analysis, suggesting room for improvement (4). There is a need to evaluate the effectiveness of intensive preoperative and/or postoperative chemotherapy with multiple agents, including some new biologic agents, in patients at high risk of relapse.

The type I HER family has 4 homologous members: HER1/erbB1 [EGF receptor (EGFR)], HER2/erbB2 (HER2), HER3/erbB3, and HER4/erbB4. All members share a

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Translational Relevance

The clinical significance of EGF receptor (EGFR) and HER2 overexpression remains to be fully defined because not all previous studies have shown an association between overexpression of these receptors and poor outcomes of patients with gastric cancer. We studied archived specimens obtained from 829 patients enrolled in the Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC) trial at 65 centers. All specimens were evaluated by standard methods and unified criteria in a central laboratory. The results provide compelling evidence that an EGFR 3+ status on immunohistochemical analysis, but not HER2 positivity, is significantly associated with poor outcomes after curative resection of stage II/III gastric cancer. There was no apparent interaction between S-1 and EGFR or HER2 status with respect to survival.

common structure, with an extracellular ligand-binding domain, a transmembrane domain, and an intracytoplasmic tyrosine kinase domain. Ligand binding to these receptors induces the formation of receptor homodimers and heterodimers, and the activation of downstream signaling pathways. The HER family might therefore contribute to malignant progression. In gastric cancers, overexpressions of EGFR and HER2 are considered prognostic factors, and have been targeted by novel biologic agents (5–10). Recently, the first phase III Trastuzumab for Gastric Cancer (ToGA) trial showed that trastuzumab enhanced the efficacy of chemotherapy in HER2-positive advanced gastric cancer, indicating that HER2 expression might predict the response to anti-HER2 agents even in gastric cancer (11). However, the clinical significance of EGFR and HER2 overexpression remains to be fully defined because not all studies have shown an association with poor outcomes (12, 13).

The present study therefore explored the protein expression of EGFR and HER2 using immunohistochemical analysis and gene amplification of *HER2* by dual-color *in situ* hybridization (dual-ISH) in gastric cancer tissues obtained from patients enrolled in the ACTS-GC. We retrospectively evaluated the impact of the expression of these receptors on treatment outcomes.

Materials and Methods

Patients and sample collection

Tumor tissue was collected from patients enrolled in the ACTS-GC. The inclusion criteria and the treatment protocol were as described previously (3, 4).

The present biomarker study was designed retrospectively after the completion of the first interim analysis of the ACTS-GC. Archived formalin-fixed, paraffin-embedded (FFPE) specimens obtained by surgical resection were available for 829 (78.3%) of the 1,059 patients who were enrolled in the ACTS-GC at 65 centers. The specimens were shipped to the National Cancer Center Hospital East (Kashiwa, Japan), where immunohistochemical and dual-ISH analyses were conducted, and the results were evaluated. The protocol of this biomarker study was approved by the ethics committee of the Japanese Gastric Cancer Association and the Institutional Review Board of each participating hospital.

IHC

All of the reagents and instruments for IHC were manufactured by Ventana Medical Systems, Inc. FFPE sections (thickness = 3–5 μm) were automatically stained with Ventana BenchMark ULTRA using primary antibodies against EGFR (CONFIRM EGFR 3C6) and HER2 (I-VIEW PATHWAY anti-HER2/neu 4B5), and a Ventana iView DAB Universal Kit, according to the manufacturer's protocol. Staining was evaluated using light microscopy and was interpreted by 2 independent pathologists (K. Kitada and A. Ochiai) who were blinded to all clinical information. Tumor cell-membrane immunostaining was scored using a 4-grade scale (0, 1+, 2+, or 3+). EGFR reactivity was scored as 0 if there was no membranous reactivity within the tumor, or as 1+, 2+, or 3+ depending on the intensity above the background level (7). We followed the consensus panel recommendations for HER2 scoring in gastric cancer (14).

Dual-ISH

All reagents and instruments for dual-ISH were manufactured by Ventana Medical Systems, Inc. Dual-ISH analyses for *HER2* were carried out for specimens with IHC scores of 2+ or 3+ with Ventana Benchmark ULTRA, using DNA cocktail probes [*HER2* and *CEP17* (centromeric probe 17)] according to the manufacturer's protocol. For each specimen, the numbers of *HER2* signals (silver ISH, black) and *CEP17* signals (red ISH, red) were counted for 20 nuclei, and the *HER2*/*CEP17* ratio was calculated by dividing the total number of *HER2* signals by the total number of *CEP17* signals. Negativity for *HER2* gene amplification was defined as an *HER2*/*CEP17* ratio of less than 1.8, whereas positivity was defined as an *HER2*/*CEP17* ratio of more than 2.2. If the *HER2*/*CEP17* ratio was in the equivocal range (1.8–2.2), the number of *HER2* and *CEP17* signals was counted for 20 additional nuclei, and the *HER2*/*CEP17* ratio was calculated from the results of 40 nuclei. Eventually, amplification of *HER2* was defined as an *HER2*/*CEP17* ratio of 2.0 or more, based on a partially modified version of the *HER2* scoring system for breast cancer (15).

Definition of positivity

For EGFR, an IHC score of 3+ was defined as positive, and IHC scores of 0, 1+, and 2+ were defined as negative. For HER2, an IHC score of 3+ or an IHC score of 2+ with a dual-ISH *HER2*/*CEP17* ratio of 2.0 or more was defined as positive, and IHC scores of 0 and 1+ or a score of IHC 2+ with a dual-ISH *HER2*/*CEP17* ratio of less than 2.0 were defined as negative (14).

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Reverse-transcription PCR

Representative hematoxylin and eosin–stained slides of FFPE specimens were reviewed by a pathologist to estimate tumor load per sample. Slide sections 10 μm in thickness were then stained with nuclear fast red (Sigma-Aldrich) for manual microdissection. Tumor tissue was selected at a magnification of 5 to 10 times and dissected from the slide using a scalpel, as described previously (16).

RNA isolation from tumor tissue and the cDNA preparation steps were conducted as described previously (17), with a slight modification in the extraction step, using RNeasy Mini Elute spin-columns (Qiagen).

Gene expression levels of *EGFR* and *HER2* were determined by means of TaqMan real-time PCR (Life Technologies) as described previously (17). β-Actin was used as an endogenous reference gene. The detection of amplified cDNA results in a cycle threshold (C_t) value, which is inversely proportional to the amount of cDNA. Gene expression values (relative mRNA levels) are expressed as ratios (differences between the C_t values) between the gene of interest (*EGFR* or *HER2*) and a reference gene (β-actin). This reference gene provides a baseline measurement for the amount of RNA isolated from a specimen.

Statistical analysis

Survival curves were estimated using the Kaplan–Meier product-limit method, and the statistical significance of differences between survival curves was assessed using the log-rank test. Univariate and multivariate survival analyses were conducted using a Cox proportional hazards model. Categorical data analysis was conducted using the χ^2 test. Either the Wilcoxon test or the Kruskal–Wallis test was used to assess correlations between groups. Results were considered statistically significant at $P < 0.05$. All statistical analyses were carried out with the SAS software package version 9.1 and JMP software version 8.01 (SAS Institute Inc.).

We estimated what minimum difference in survival would be required with EGFR- or HER2-positive cancers to show a survival difference as compared with EGFR- or HER2-negative cancers, respectively. We assumed that patients with EGFR- or HER2-positive tumors would have poorer outcomes. Given a positivity rate of 10%, 15%, or 20%, demonstration of a statistically significant difference in survival between patients with positive tumors and those with negative tumors would require HRs of at least 1.624, 1.520, and 1.465, respectively, assuming a 2-sided $\alpha = 0.05$ and a power = 80% in a proportional hazards model.

Results

Patients and sample collection

When the biomarker population of this study was compared with the total population of ACTS-GC as previously reported (3), there was no significant difference between these groups (Table 1). The IHC results were obtained for both EGFR and HER2 expression in all 829 specimens as follows: EGFR grade 0, 204 (24.6%); EGFR grade 1+, 372 (44.9%); EGFR grade 2+, 178 (21.5%); EGFR grade 3+, 75 (9.0%); HER2 grade 0, 443 (53.4%); HER2 grade 1+, 210 (25.3%); HER2 grade 2+, 101 (12.2%); and HER2 grade 3+, 75 (9.0%). Representative examples of immunostaining for EGFR and HER2 are shown in Supplementary Fig. S1 and S2.

Dual-ISH analyses were conducted on 176 specimens with a HER2 IHC score of 2+ or 3+. The IHC score and dual-ISH status for HER2 were as follows: IHC 2+/dual-ISH negative, 63 (7.6%); IHC 2+/dual-ISH positive, 38 (4.6%); IHC 3+/dual-ISH negative, 2 (0.2%); and IHC 3+/dual-ISH positive, 72 (8.7%). Dual-ISH could not be determined in one specimen, but this was classified as HER2-positive because the IHC score was 3+. IHC 3+ scores were generally consistent with dual-ISH positive status (72/74 cases; 97.3%), whereas IHC 2+ scores were not (38/101 cases; 37.6%).

We also measured the relative gene-expression levels of *EGFR* and *HER2* by reverse-transcription PCR (RT-PCR) analysis in tumor tissue dissected from FFPE specimens. The IHC scores for EGFR and HER2 significantly correlated with their gene-expression levels ($P < 0.001$, Kruskal–Wallis test; Supplementary Fig. S3).

Eventually, we classified 75 cases (9.0%) as positive for EGFR and 113 (13.6%) as positive for HER2. The groups were well balanced with respect to EGFR and HER2 status and other factors (Table 1). Both EGFR and HER2 positivities were more common among differentiated type than undifferentiated type tumors (EGFR, 58.7%, $P < 0.001$; HER2, 75.2%, $P < 0.001$ [χ^2-test]). HER2 positivity was associated with male gender ($P < 0.001$), older age ($P = 0.0052$), and lower tumor stage ($P < 0.001$), whereas EGFR positivity was not (Supplementary Table S1). Eighteen cases (2.2%) were positive for both EGFR and HER2, 57 (6.9%) were positive for EGFR alone, and 95 (11.5%) were positive for HER2 alone.

Effects of EGFR and HER2 expressions on survival

Five-year OS and relapse-free survival (RFS) were 73.6% [95% confidence interval (CI) = 69.3%–77.9%] and 66.7% (95% CI = 62.1%–71.3%), respectively, in the S-1 group, compared with 61.9% (95% CI = 57.1%–66.7%) and 53.7% (95% CI = 48.8%–58.7%) in the surgery-only group, respectively. These figures were similar to the ACTS-GC 5-year follow-up data (4).

EGFR-positive status was significantly associated with worse outcomes in the study group as a whole (Table 2, Fig. 1A; Kaplan–Meier curves for the OS of patients according to the EGFR IHC score are shown in Supplementary Fig. S4). The results for 5-year RFS were similar to those for 5-year OS (Table 2). EGFR-positive status was also associated with worse outcomes in both the S-1 group and the surgery-only group (Table 2). Irrespective of EGFR status, the 5-year OS in the S-1 group was longer than that in the surgery-only group (Fig. 1B and C).

In contrast, there was no correlation between HER2 status and patient outcomes in the study group as a whole (Table 2, Fig. 2A). The 5-year RFS was similar to the 5-year OS (Table 2). HER2-positive status was not associated with outcomes in either the S-1 group or the surgery-only group

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Table 1. Characteristics of the patients

	Entire population of ACTS-GC			Biomarker study population of ACTS-GC		
	S-1 ($n = 529$)	Surgery only ($n = 530$)	*P* value[a]	S-1 ($n = 415$)	Surgery only ($n = 414$)	*P* value[a]
Sex, *n* (%)			0.98			0.90
Male	367 (69.4)	369 (69.6)		282 (68.0)	283 (68.4)	
Female	162 (30.6)	161 (30.4)		133 (32.0)	131 (31.6)	
Age, *n* (%)			0.86			0.72
<60	199 (37.6)	195 (36.8)		160 (38.6)	158 (38.2)	
60–69	193 (36.5)	215 (40.6)		149 (35.9)	161 (38.9)	
70–80	137 (25.9)	120 (22.6)		106 (25.5)	95 (22.9)	
Median, y	63	63		63	62	
Range, y	27–80	33–80		27–80	33–80	
Tumor stage, *n* (%)			0.81			0.93
T1	1 (0.2)	0 (0)		1 (0)	0 (0)	
T2	289 (54.6)	286 (54.0)		222 (53.5)	223 (53.9)	
T3	225 (42.5)	232 (43.8)		180 (43.4)	182 (44.0)	
T4	14 (2.6)	12 (2.3)		12 (2.9)	9 (2.2)	
Nodal stage, *n* (%)[b]			0.72			0.52
N0	51 (9.6)	64 (12.1)		40 (9.6)	52 (12.6)	
N1	296 (56.0)	281 (53.0)		233 (56.1)	222 (53.6)	
N2	182 (34.4)	185 (34.9)		142 (34.2)	140 (33.8)	
N3	0 (0)	0 (0)		0 (0)	0 (0)	
Lymph-node metastases, *n* (%)		0.37			0.18	
0	51 (9.6)	64 (12.1)		40 (9.6)	52 (12.6)	
1–6	331 (62.6)	325 (61.3)		254 (61.2)	254 (61.4)	
7–15	117 (22.1)	113 (21.3)		97 (23.4)	85 (20.5)	
≥16	30 (5.7)	28 (5.3)		24 (5.8)	23 (5.6)	
Cancer stage, *n* (%)[c]			0.78			0.48
II	236 (44.6)	238 (44.9)		183 (44.1)	189 (45.7)	
IIIA	202 (38.2)	207 (39.1)		159 (38.3)	162 (39.1)	
IIIB	90 (17.0)	85 (16.0)		73 (17.6)	63 (15.2)	
IV	1 (0.2)	0 (0)		0 (0)	0 (0)	
Histologic type, *n* (%)[d]			0.73			0.91
Differentiated	214 (41.6)	209 (40.3)		166 (40.0)	166 (40.1)	
Undifferentiated	301 (58.4)	307 (59.7)		249 (60.0)	245 (59.2)	
EGFR status, *n* (%)			—			0.54
Negative	—	—		380 (91.6)	374 (90.3)	
Positive	—	—		35 (8.4)	40 (9.7)	
HER2 status, *n* (%)			—			0.77
Negative	—	—		357 (86.0)	359 (86.7)	
Positive	—	—		58 (14.0)	55 (13.3)	

NOTE: Characteristics of the patients in entire population of ACTS-GC was referred by ref. 3.

[a]*P* values for sex, EGFR status, and HER2 status were calculated with the use of the χ^2 test. *P* values for age, tumor stage, nodal stage, number of lymph-node metastases, cancer stage (Japanese classification), and histologic type were calculated with the use of the Wilcoxon test.

[b]Nodal stages according to the Japanese classification were defined as follows: N0, no evidence of lymph node metastasis; N1, metastasis to group 1 lymph nodes; N2, metastasis to group 2 lymph nodes; N3, metastasis to group 3 lymph nodes. Groups 1, 2, and 3 are regional lymph node classifications defined according to the location of the primary tumor and are based on the results of studies of lymphatic flow at various tumor sites and the observed survival associated with metastasis at each nodal station (i.e., position in relation to primary node).

[c]Cancer stages according to the Japanese classification were defined as follows: stage IA, T1N0; stage IB, T1N1 or T2N0; stage II, T1N2, T2N1, or T3N0; stage IIIA, T2N2, T3N1, or T4N0; stage IIIB, T3N2 or T4N1; stage IV, T4N2, any T stage with N3, or distant metastasis.

[d]In entire population of ACTS-GC, histologic type was classified among eligible patients ($n = 1,034$). In the surgery-only group of biomarker study population, cancers could not be classified as differentiated or undifferentiated in 3 patients.

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Table 2. Univariate analysis of OS and RFS according to the status of EGFR and HER2

				OS			RFS		
Marker	Group	Status	Number of patients	5-year survival (%)	HR (95% CI)	*P* value (log-rank)	5-year survival (%)	HR (95% CI)	*P* value (log-rank)
EGFR									
	All	Negative	754	69.0	1		61.3	1	
		Positive	75	55.4	1.642 (1.139–2.366)	0.007	49.9	1.451 (1.030–2.045)	0.033
	S-1	Negative	380	74.9	1		68.2	1	
		Positive	35	60.0	1.787 (1.018–3.134)	0.043	51.4	1.773 (1.066–2.950)	0.027
	Surgery only	Negative	374	63.1	1		54.3	1	
		Positive	40	51.2	1.514 (0.936–2.449)	0.091	48.7	1.219 (0.767–1.939)	0.402
HER2									
	All	Negative	716	68.3	1		60.0	1	
		Positive	113	64.5	1.155 (0.822–1.624)	0.406	62.3	0.991 (0.716–1.371)	0.955
	S-1	Negative	357	74.2	1		66.5	1	
		Positive	58	69.9	1.170 (0.697–1.965)	0.552	68.2	1.000 (0.609–1.643)	1.000
	Surgery only	Negative	359	62.4	1		53.5	1	
		Positive	55	58.8	1.167 (0.742–1.833)	0.504	56.0	0.997 (0.649–1.530)	0.988

(Table 2). Similarly, there was no correlation between the 75 patients with IHC 3+ and patient outcomes (5-year OS in the IHC 3+ and in the IHC 0/1+/2+ were respectively 64.7% and 68.1%, HR = 1.178, 95% CI = 0.807–1.720, log-rank $P = 0.396$; and 5-year RFS in the IHC 3+ and in the IHC 0/1+/2+ were respectively 62.2% and 60.1%, HR = 0.942, 95% CI = 0.625–1.418, log-rank $P = 0.773$). Irrespective of HER2 status, the 5-year OS in the S-1 group was longer than that in the surgery-only group (Fig. 2B and C).

Multivariate analysis in overall study population

The prognostic relevance of EGFR and HER2 was assessed using a multivariate proportional hazards model adjusted for the following established clinical prognostic factors: treatment arm, gender, age, cancer stage (Japanese classification of gastric carcinoma, 2nd English edition; ref. 18), and histologic type (Table 3). Although treatment arm and cancer stage were the strongest prognostic factors, EGFR status was also an independent prognostic factor.

Subgroup analysis

The OS in the study group as a whole was analyzed according to gender, age, cancer stage, histologic type, and EGFR/HER2 status; no interaction was found between S-1 treatment and any of these factors (Fig. 3). Kaplan–Meier estimates of OS plotted according to EGFR (Fig. 1B and C) and HER2 status (Fig. 2B and C) revealed that S-1 treatment improved survival irrespective of EGFR or HER2 status.

Discussion

The present study retrospectively evaluated the influence of EGFR and HER2 expression on the outcomes of patients enrolled in the ACTS-GC. EGFR positivity was found to be associated with worse outcomes, in agreement with earlier findings (5–7, 9). Although most previous studies defined EGFR positivity as an IHC score of 2+ and 3+, no consensus definition has been reached. To the best of our knowledge, this is the first study to show that EGFR IHC 3+ status correlates significantly with poor outcome in patients with gastric carcinoma.

Kim and colleagues reported a similar distribution of EGFR protein-expression IHC scores to those of the present study in 511 specimens of gastric carcinoma tissue (7). They also reported that 13 (61.9%) of 21 cases with IHC scores of 3+ showed *EGFR* gene amplification or high polysomy on FISH, whereas this was observed in only 14 (11.8%) of 119 cases with scores of 2+. Our present study confirmed that the EGFR IHC scores significantly correlated with *EGFR* gene-expression levels. Moreover, the median *EGFR* gene expression for cases with IHC scores of 3+ was higher than that for cases with scores of 0, 1+, and 2+ (Supplementary Fig. S3A), suggesting that a score of 3+ could be a new criterion for defining EGFR positivity in gastric cancer. This was strongly linked to EGFR overexpression and poor outcomes for patients with gastric carcinoma in this study (Supplementary Fig. S4).

Multivariate analysis revealed that an IHC score of EGFR 3+ was an independent predictor of unfavorable outcomes. As well as being a prognostic marker, EGFR positivity might be a predictor of response to EGFR-targeted therapy in gastric cancer. A phase II study showed a significant association between increased *EGFR* gene copy number ($\geq$4.0) and OS in a subset of patients with gastric and esophagogastric junction cancer who received cetuximab combined with oxaliplatin/leucovorin/5-fluorouracil (19, 20). In addition, among 58 patients with metastatic colorectal carcinoma (mCRC) who received panitumumab in a previous study, 6 of 20 patients with an *EGFR* gene copy number more than 2.47 had an objective response, whereas no tumor response was observed in patients with copy numbers below this ($P = 0.0009$; ref. 21). Similarly, an increased *EGFR* copy number was significantly associated with response to cetuximab therapy in patients with mCRC (22), although the relationship between EGFR

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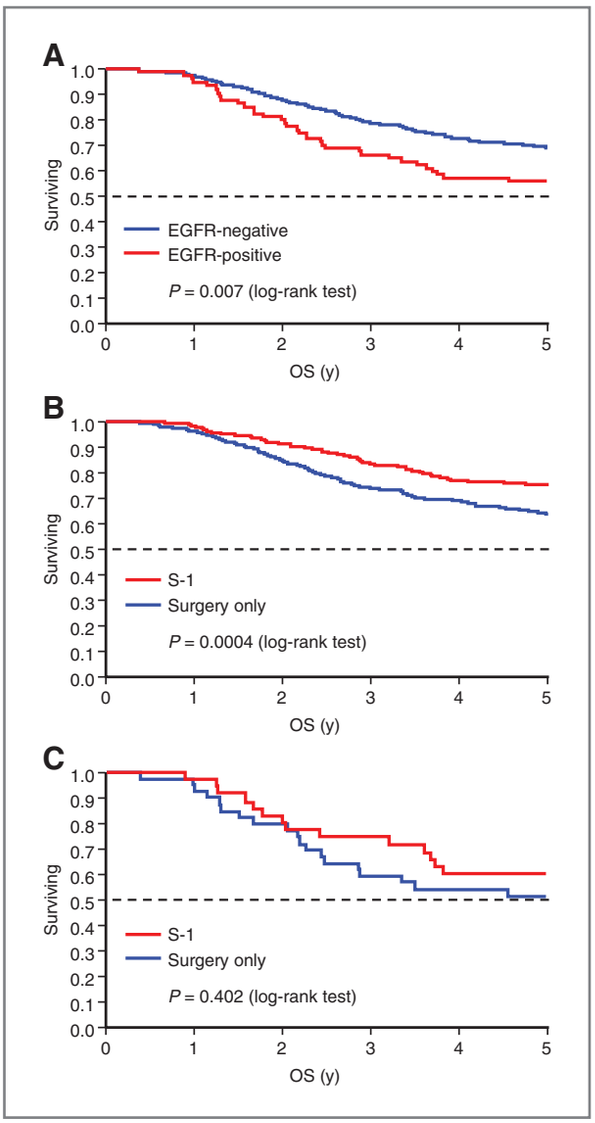

Figure 1. Kaplan–Meier curves for OS according to EGFR status. For EGFR, an IHC score of 3+ was defined as positive, and IHC scores of 0, 1+, and 2+ were defined as negative. A, OS for all patients ($n = 829$): EGFR-negative ($n = 754$) versus EGFR-positive ($n = 75$). B, OS for patients with EGFR-negative tumors: S-1 group ($n = 380$) versus surgery-only group ($n = 374$). C, OS for patients with EGFR-positive tumors: S-1 group ($n = 35$) versus surgery-only group ($n = 40$).

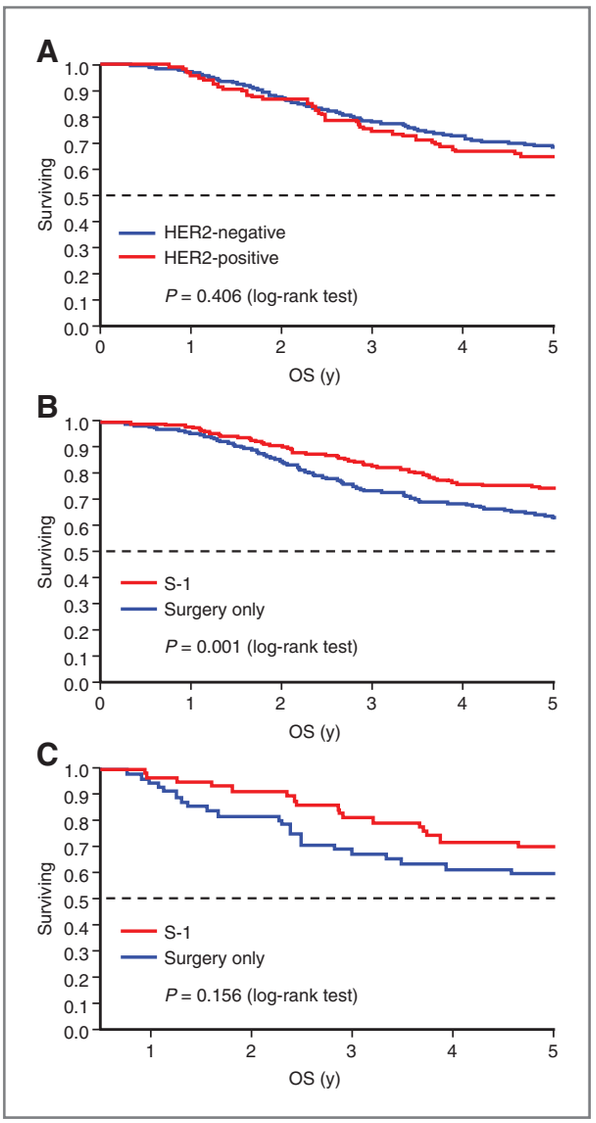

Figure 2. Kaplan–Meier curves for OS according to HER2 status. A, OS for all patients ($n = 829$): HER2-negative ($n = 716$) versus HER2-positive ($n = 113$). B, OS for patients with HER2-negative tumors: S-1 group ($n = 357$) versus surgery-only group ($n = 359$). C, OS for patients with HER2-positive tumors: S-1 group ($n = 58$) versus surgery-only group ($n = 55$).

overexpression on IHC and the response to cetuximab remains controversial (23).

Although *KRAS* mutation status is used as a negative predictive marker for EGFR-targeted agents in colorectal cancer, the frequency of *KRAS* mutations in gastric cancer seems to be relatively low (3%–21%; ref. 24). Several phase III trials of combined chemotherapy with EGFR-targeted agents, such as cetuximab, panitumumab, and lapatinib are ongoing in patients with unresectable advanced gastric cancer (10); detailed information on alterations of the EGFR protein or gene in these trials is needed to predict the response to anti-EGFR therapy in gastric cancer more accurately (19).

The frequency of EGFR overexpression on IHC in gastric carcinoma ranges from 2% to 30% (7, 8, 25). Possible reasons for this wide variation include differences in fixation techniques, antibodies, scoring systems, subjectivity of pathologist interpretation, and intratumoral staining heterogeneity. To improve the accuracy of assessing EGFR positivity, additional gene-amplification analysis might be useful, as conducted for HER2, and standardized EGFR testing procedures should be established.

The prevalence of HER2 overexpression on IHC in the present study fell within the previously reported range (median positive rate = 18%; range = 4%–53%; ref. 12).

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Table 3. Cox regression multivariate analysis of prognostic factors for OS in all patients

Factor	Group	Number of patients	5-year survival (%)	HR (95% CI)	*P* value
Arm	Surgery only	414	61.9	1	
	S-1	415	73.6	0.617 (0.481–0.790)	<0.001
Sex	Male	565	67.2	1	
	Female	264	69.0	0.988 (0.757–1.301)	0.932
Age, y	<60	318	69.5	1	
	60–69	310	72.2	1.242 (1.057–1.460)	
	70–80	201	58.4	1.544 (1.118–2.132)	0.009
Cancer stage (Japanese classification)	II	372	77.0	1	
	IIIa	321	63.7	1.683 (1.431–1.979)	
	IIIb	136	52.2	2.833 (2.048–3.918)	<0.001
Histologic type	Differentiated	332	65.1	1	
	Undifferentiated[a]	497	69.6	0.894 (0.684–1.171)	0.412
EGFR status	Negative	754	69.0	1	
	Positive	75	55.4	1.504 (1.020–2.149)	0.040
HER2 status	Negative	716	68.3	1	
	Positive	113	64.5	1.068 (0.736–1.514)	0.722

[a]Including 3 patients with gastric cancer categorized into neither differentiated nor undifferentiated type.

Consistent with the results of Begnami and colleagues (8), the concordance between IHC (scores 2+ and 3+) and dual-ISH (positive) was 62.9% in the present study; most IHC 3+ results corresponded with dual-ISH positive status (98.6%), whereas IHC 2+ tumors showed relatively low concordance between IHC score and dual-ISH status (37.6%). The present results are also in agreement with the finding that HER2 positivity is more prevalent among differentiated-type tumors than undifferentiated-type tumors (6, 8, 11). Consequently, we consider our present evaluation of HER2 status to be realistic.

The role of HER2 as a prognostic factor in gastric cancer remains controversial. A recent systematic review assessing the impact of HER2 overexpression on survival found that 20 studies (57%) reported no difference in OS, 2 (6%) showed significantly longer OS in patients with HER2 overexpression, and 13 (37%) found significantly worse OS in patients with HER2 overexpression (12). To the best of our knowledge, the present investigation is the first large biomarker study to evaluate the influence of HER2 positivity on the postoperative outcomes of patients with gastric cancer enrolled in a randomized phase III trial. Trastuzumab was not administered to these patients until the completion of the 5-year follow-up, because it had not been approved at that time. The present results therefore provide strong evidence that HER2 status does not influence outcomes after D2 dissection for locally advanced gastric cancer in East Asian patients, in contrast to breast cancer.

Although it is unclear why EGFR overexpression was a prognostic marker in this study and HER2 overexpression was not, it might be partially explained by the fact that gastric cancer is a heterogeneous disease. A recent study reported that patients with HER2-positive gastric tumors have longer OS than those with HER2-negative tumors. This finding was based on an analysis of 381 patients with metastatic gastric/gastroesophageal junction cancer. On subgroup analysis, similar differences in OS according to HER2 status were seen in the subgroup of patients with intestinal-type cancer but not in those with diffuse-type cancer (26). Because the subgroup of patients with intestinal-type cancer includes a higher proportion of HER2-positive cases than EGFR-positive cases, as shown in Table 2, the association between intestinal-type and good outcomes may mask potential prognostic effects of HER2 positivity. Further understanding of the molecular biologic and pathologic characteristics of gastric cancer is considered necessary to improve EGFR and HER2 targeting in this disease.

Neither EGFR nor HER2 was associated with the efficacy of S-1; this was not surprising because neither one is thought to have an appreciable impact on the metabolism or mechanism of action of S-1. In several preclinical studies on mice, the antitumor activity of S-1 combined with trastuzumab, lapatinib, or cetuximab was greater than that of either drug alone on xenografts of gastric cancer cells overexpressing HER2 or EGFR. This enhancement of activity was considered to be mediated by thymidylate synthase (27, 28). These experimental results suggest that S-1 combined with an EGFR- or HER2-targeted agent (or both) is a promising regimen for patients with EGFR/HER2-positive gastric cancer.

In conclusion, the current study provides compelling evidence that EGFR 3+ status, but not HER2 status, on IHC is significantly associated with worse patient outcomes after curative resection of stage II/III gastric cancer. Furthermore, there is no apparent interaction between S-1 and EGFR or HER2 status with respect to survival. We therefore propose

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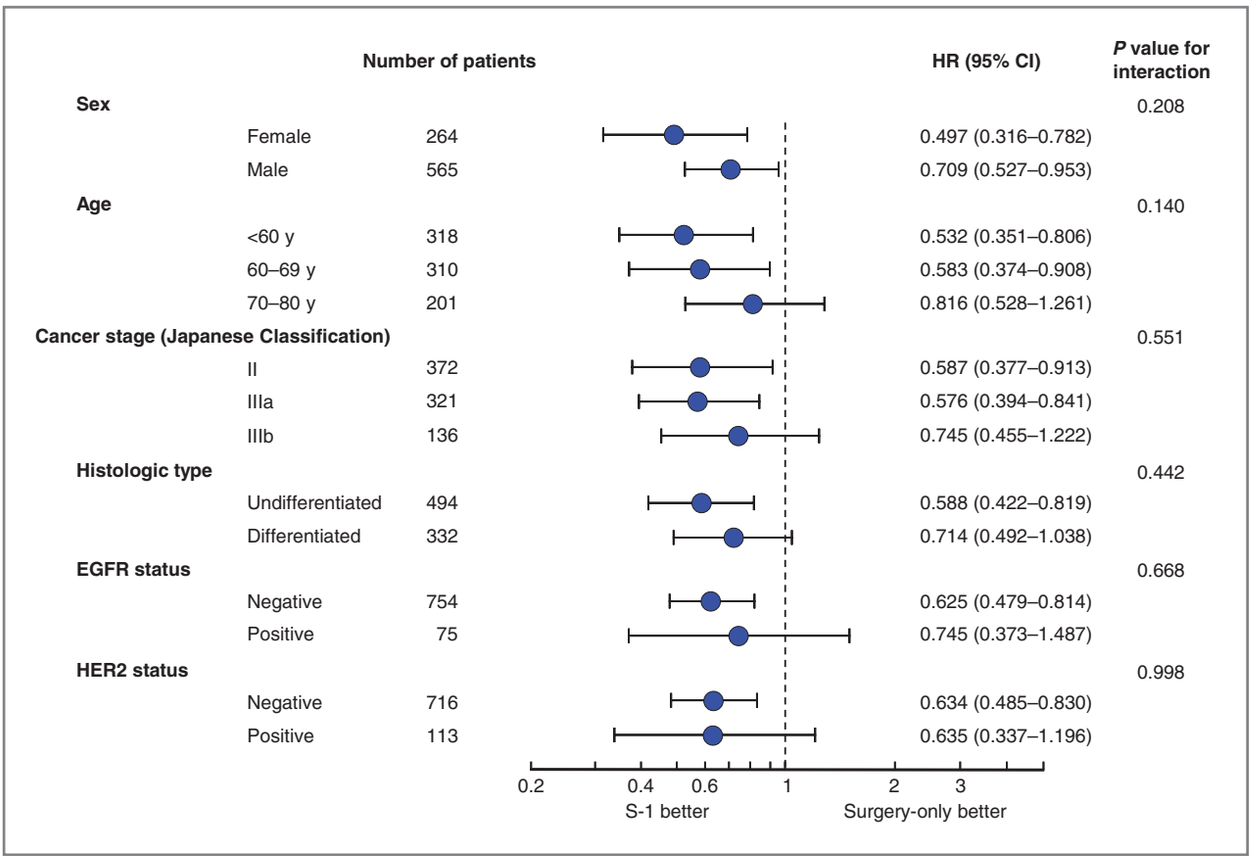

Figure 3. Subgroup analysis for OS. In the surgery-only group, cancers could not be classified as differentiated or undifferentiated in 3 patients.

that EGFR status should be evaluated in future clinical trials of EGFR-targeted agents. S-1 combined with EGFR/HER2-targeted agents merits further investigation in patients with gastric cancer.

Disclose of Potential Conflicts of Interest

A. Ochiai: commercial research grant, Taiho Pharmaceutical Co.; Ltd., other commercial research support, Chugai; and consultant/advisory board, Roche Diagnostic. W. Ichikawa: honoraria from speakers bureau, Taiho Pharmceutical Co., Ltd. H. Katai: commercial research grant and honoraria from speakers bureau, Taiho Pharmaceutical Co. Ltd. T. Sano: honoraria from speakers bureau, Taiho Pharmaceutical Co. Ltd. and Chugai Pharmaceutical. The funding source of this study had no role in the study design, data collection, data analysis, or interpretation.

Authors' Contributions

Conception and design: M. Terashima, K. Kitada, A. Ochiai, W. Ichikawa, M. Sasako
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Kitada, A. Ochiai, S. Sakuramoto, H. Katai, T. Sano, H. Imamura, M. Sasako
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Kitada, H. Katai, T. Sano, M. Sasako
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