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ORIGINAL ARTICLE

Evaluation of HER2-based biology in 1,006 cases of gastric cancer in a Japanese population

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Abstract
Background The ToGA trial demonstrated the beneficial effect of trastuzumab in gastric cancer patients with human epidermal growth factor receptor 2 (HER2)-overexpressing tumors. Therefore, evaluation of the relationship between HER2 expression and gastric cancer biology using a validated system has become an even more important task. Herein, we verified the correlation between HER2 overexpression in the tumor and the clinical course of gastric cancer patients.
Methods A total of 1,006 consecutive patients with gastric cancer who underwent surgery at the National Cancer Center Hospital East between January 2003 and July 2007 were examined using the tissue microarrays approach. HER2 expression was determined based on an immunohistochemistry score of 3+, or an immunohistochemistry score of 2+ plus HER2 gene amplification as detected by double-color fluorescent in situ hybridization. A retrospective review of the medical records was conducted to determine the correlation between the presence of HER2 overexpression and clinicopathological factors. Then, in 948 patients who had undergone curative resection, HER2 status was compared with the survival.
Results HER2 overexpression was detected in 118 (11.7 %) patients. HER2 overexpression was correlated with age, gender, grade of differentiation, expanding growth pattern, and nodal status. In the survival analysis, HER2 overexpression was not found to be correlated with either disease-specific survival or recurrence-free survival.
Conclusions HER2 overexpression in the tumor was not identified as a significant prognostic factor in patients with operable gastric cancer. The HER2-targeted therapy may be beneficial in a proportion of cases.

Keywords Gastric cancer · HER2

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Introduction

Overexpression of the human epidermal growth factor receptor 2 (HER2) protein has been detected in various cancers [1]. With regard to breast cancer, approximately two decades have elapsed since HER2 was functionally implicated in the pathogenesis. At present, an HER2-based concept of tumor biology has been established, and trastuzumab (Herceptin, Genentech/Roche), a monoclonal humanized antibody directed against HER2, is a pivotal agent for the management [2]. In gastric cancer also, many publications have suggested a similar role of HER2 [3–8]. However, validated methods and scoring systems for evaluation of the HER2 status have remained inconsistent. This discordance has been attributed to the heterogeneous patterns of expression and incomplete membrane immunoreactivity in HER2-positive gastric cancer cells. Therefore, the clinical significance and prognostic value of HER2 have remained controversial. Recently, a new assessment of the HER2 scoring system for gastric cancer was proposed by Hofmann and colleagues [9], and subsequently, a

randomized international phase III clinical trial, ToGA, was performed using modified Hofmann's criteria. The trial revealed impressive benefits of the addition of trastuzumab to a cisplatin plus fluoropyrimidine chemotherapy doublet in patients with HER2-overexpressing gastric cancers [10]. Therefore, evaluation of the relationship between HER2 expression and gastric cancer biology using a validated scoring system has become an even more important task. Herein, we verified the correlation between tumor HER2 overexpression and the clinical course of gastric cancer using the tissue microarrays (TMA) approach.

Patients and methods

Patients and disease staging

A total of 1,006 consecutive patients with gastric cancer who underwent surgery at the National Cancer Center Hospital East between January 2003 and July 2007 were examined for the present study. The medical records and surgical specimens of these patients were retrospectively evaluated after obtaining approval from the Investigational Review Board of the National Cancer Center. The disease stage was determined according to the International Union Against Cancer (UICC)-TNM classification (seventh edition) [11]. Neo-adjuvant and adjuvant chemotherapy was administered in 54 (5.4 %) and 67 (6.7 %) patients, respectively.

Tissue microarray and immunohistochemistry

Representative tumor areas were selected and marked on hematoxylin and eosin (H&E)-stained slides for the construction of microarrays. Duplicate cylindrical cores with a diameter of 2.0 mm were prepared from the same tissue block for each case using a manual tissue arrayer (Azumaya Ika Kikai, Tokyo, Japan) and assembled in a tissue microarray format. Serial 4 μm sections were used for immunohistochemical staining. The reliability of tissue microarrays for the evaluation of HER2 gene amplification has been demonstrated in breast cancer [12]. Immunohistochemical staining was performed using the fully automated Ventana Benchmark ULTRA (Roche Diagnostics) device. Sections were dewaxed, then subjected to pretreatment with CC1 for 30 min. Then, sections were washed with reaction buffer followed by incubation with the rabbit monoclonal primary antibody HER2/neu (Clone 4B5; Roche Diagnostics) for 28 min. On-board detection using the ultraView Universal DAB kit (Roche Diagnostics), used in accordance with the manufacturer's recommendations, was used to detect the location of the primary antibody HER2 followed by counterstaining with hematoxylin 11 (Roche Diagnostics). Tissue from gastric cancer in which HER2 gene amplification had been detected by in situ hybridization (ISH) was used as controls.

Procedure for double-color ISH and evaluation of HER2 gene amplification

HER2 gene amplification was detected by the double-color ISH technique using the Ventana Benchmark ULTRA and a fully automated INFORM HER2 Dual ISH DNA Probe Cocktail assay (Roche Diagnostics). The sections were deparaffinized and pretreated with CC2 buffer (Roche Diagnostics) using high heat. An ISH-protease 3 (Roche Diagnostics) was added to the sections for 8 min for enzyme digestion of protein. A cocktail of an HER2 dinitrophenyl (DNP)-labeled probe and Chr17 digoxigenin (DIG)-labeled probe was dropped on to the sections, followed by incubation for 6 h. To detect the HER2 probe, the ultraView silver in situ hybridization (SISH) DNP Kit (Roche Diagnostics) was used. After the SISH signals were developed, the ultraView Red ISH DIG detection Kit (Roche Diagnostics) was used for detection of the Chr17 probe. Sections were counterstained with hematoxylin II (Roche Diagnostics) for 8 min and post counterstained with Bluing reagent (Roche Diagnostics) for 4 min.

The HER2 and Chr17 signals were counted in 40 nuclei at the hotspot by immunohistochemical staining. A discrete signal was counted as a single copy of HER2 or Chr17. A cluster was defined as numerous overlapping SISH signals in the nuclei that could not be detected individually. According to the manufacturer's guide, clusters were subdivided into small and large clusters using the size of a single signal as reference. Then, a small cluster was counted as 6 signals and a large cluster as 12 signals. The HER2 gene expression was classified as nonamplified if the HER2/Chr17 ratio was <2.0 and as amplified if the HER2/Chr17 ratio was ≥ 2.0.

Evaluation of HER2 expression

HER2 overexpression was determined using the proposed scoring scheme in the relevant subgroup analysis of the ToGA trial [10]. Immunohistochemically stained full-face sections from each of the TMA blocks were digitized using the Slide Path and the Nano Zoomer Digital Pathology (NDP) System (Hamamatsu, Welwyn Garden City, UK). Approximately 7 min were required to scan a slide at a resolution of 40×. Two individuals (M.A. and K.K.), who were blind to the clinical data, reviewed the digital images. Evaluation and scoring of the HER2 protein expression was performed according to Hofmann's criteria. This scoring system, described below, has been validated for use in

gastric cancer: 0 = staining or membrane reactivity in <10 % of cancer cells; 1+ = faint membrane reactivity in >10 % of cancer cells or cancer cells with reactivity in only a part of the cell membrane; 2+ = weak or moderate complete or basolateral membrane staining in >10 % of cancer cells; and 3+ = strong complete or basolateral membrane staining in >10 % of cancer cells [9]. An immunohistochemistry score of 3+, or immunohistochemistry score of 2+ plus HER2 gene amplification as detected by double-color fluorescent in situ hybridization, was defined as overexpression of HER2. Consequently, the patients in whom either of the duplicate cores was estimated as showing overexpression of HER2 were determined to be HER2 positive.

Survival analysis

The median (range) follow-up period of the surviving cases was 60.9 (1–105.5) months. The survival analysis was performed in 948 patients who undergone no residual cancer (R0) resection. Disease-specific survival (DSS) time in the patients was defined as the interval between the date of surgery and the date of cancer-related death or the date of last contact. The duration of recurrence-free survival (RFS) was calculated from the date of the operation to the date on which the first recurrence was diagnosed.

Statistics

The estimated HER2 scores of each case were compared in relationship to the demographics and tumor-related factors. Survivals were estimated using the Kaplan–Meier method, and differences were determined using a log-rank test. Fisher's exact test and Cramér's measure of association were used for comparison of the covariates between the patient groups with and without tumor HER2 overexpression. The Cox proportional hazard model and log-rank test were used for the univariate analysis performed to identify factors influencing disease-specific survival and disease-free survival in patients who underwent curative resection. The significance level was set at $p < 0.05$. All statistical analyses were performed using Dr. SPSS II for Windows (SPSS Japan, Tokyo, Japan).

Results

Demographic characteristics

In the 1,006 cases enrolled in the study, the median age of the patients at diagnosis was 64 years (range 18–92 years); the patients were all Japanese. The demographics and tumor-related factors are summarized in Table 1. Of the

Table 1 Patient demographics and tumor-related factors in 1,006 patients with gastric cancer

Gender, *n* (%)	
Male/female	677/329
Age, years	
Median/range	64/18–92
Histological features, *n* (%)	
Papillary	23 (2.3)
Tubular	472 (46.9)
Poorly differentiated	352 (35.0)
Signet ring cell	137 (13.6)
Mucinous	21 (2.1)
Mixed endocrine and tubular	1 (0.1)
Tumor location, *n* (%)	
Esophagogastric junction	31 (3.1)
Proximal third of stomach	221 (22.0)
Middle third of stomach	472 (46.9)
Distal third of stomach	282 (28.0)
Macroscopic tumor type, *n* (%)	
Type 0	521 (51.8)
Type 1	27 (2.7)
Type 2	118 (11.7)
Type 3	246 (24.5)
Type 4	77 (7.6)
Type 5	17 (1.7)
Pathological depth of penetration, *n* (%)	
T1[a]	505 (50.2)
T2[a]	127 (12.6)
T3[a]	232 (23.1)
T4[a]	142 (14.1)
Pathological nodal status, *n* (%)	
N0[b]	609 (60.5)
N1[b]	141 (14.0)
N2[b]	100 (9.9)
N3a[b]	88 (8.7)
N3b[b]	60 (6.0)
Nx[b]	8 (0.8)
Pathological TMN stage, *n* (%)	
Stage I	544 (54.1)
Stage II	213 (21.1)
Stage III	188 (18.8)
Stage IV	61 (6.1)
Surgical procedure, *n* (%)	
Total gastrectomy	276 (27.4)
Distal gastrectomy	650 (64.6)
Proximal gastrectomy	75 (7.5)
Partial resection of stomach	5 (0.5)
Resection margin, *n* (%)	
R0[c]	948 (94.2)
R1[c]	26 (2.6)
R2[c]	32 (3.2)

Table 1 continued

Neo-adjuvant chemotherapy, *n* (%)	
Present	54 (5.4)
Absent	952 (94.6)
Adjuvant chemotherapy, *n* (%)	
Present	67 (6.7)
Absent	939 (93.3)

[a] T, primary tumor

[b] N, regional lymph node

[c] R, residual tumor

total, 495 (49.2 %) patients were classified into the well-differentiated and 489 (48.6 %) into the poorly differentiated tumor category. Mucinous carcinoma was diagnosed in 21 (2.1 %) patients; there was 1 case of mixed endocrine and tubular adenocarcinoma. Although gastrectomy and systematic D2 regional lymph node dissection with curative intent was performed in 952 (94.6 %) patients, 4 were found to be microscopically margin positive. Splenectomy was added to total gastrectomy in patients when the tumor was located on the greater curvature of the stomach and where the tumor extended further than the submucosal layer of the stomach. Palliative resection was performed in 54 (5.4 %) patients. The median number of dissected lymph nodes and number of lymph nodes with metastasis were 37 (range 6–118) and 0 (range 0–68), respectively.

Although 127 patients died of gastric cancer, 28 patients died of other disease. Recurrence was observed in 152 patients. In the 948 patients who had undergone R0 resection, both median DSS and median RFS were longer than 5 years, and the cumulative 5-year disease-specific survival (5Y-DSS) rate and 5-year recurrence-free survival (5Y-RFS) rate were 84.5 and 83.3 %, respectively. Of the total of 1,006 patients, the 3Y-DSS and 5Y-DSS of the patients analyzed by the disease stage were as follows: 99.2 and 98.1 % in stage I patients, 91.2 and 86.5 % in stage II patients, and 68.1 and 61.0 % in stage III patients, respectively. The 3Y-DSS of the patients with stage IV disease was 23.2 %.

HER2 scores

Immunohistochemical analysis was performed to examine the expression of HER2 in all the cases (Fig. 1). The numbers of patients with an HER2 score of 0, 1+, 2+, and 3+ were 455 (45.2 %), 360 (35.8 %), 94 (9.3 %), and 97 (9.6 %), respectively. Concordance of the immunohistochemistry results for HER2 protein was evaluated between duplicate samples. The concordance rates for immunohistochemistry scores of 2+/3+ and 0/1+ between the samples were 74.5 and 94.7 %, respectively. In 94 patients

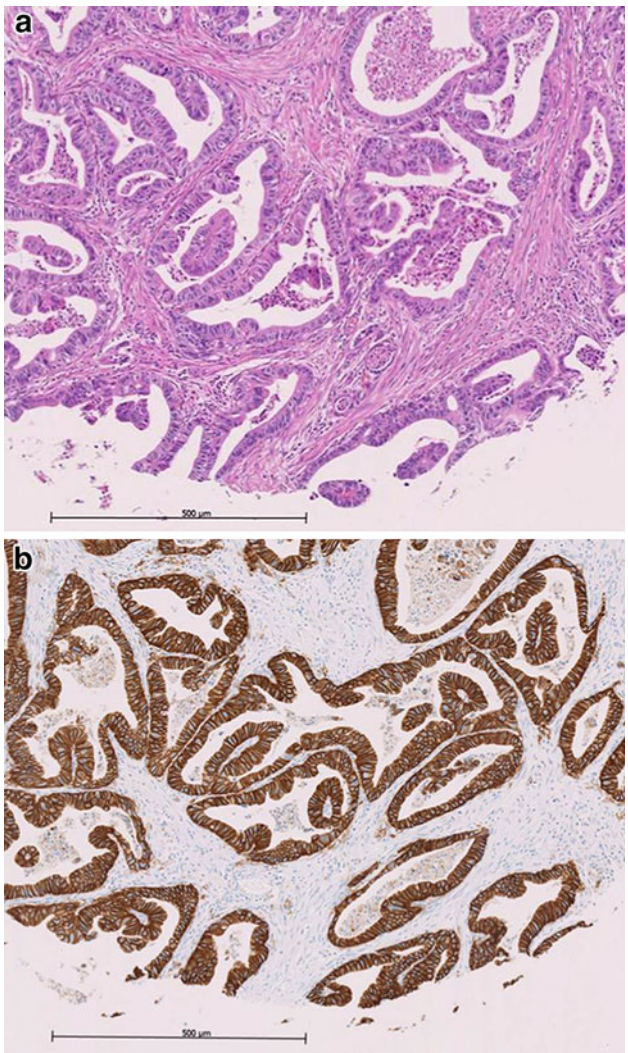

Fig. 1 Representative photomicrograph of a human epidermal growth factor receptor 2 (HER2) 3+ specimen. **a** Hematoxylin and eosin (H&E) staining shows a typical tubular arrangement of well-differentiated tumor cells. **b** Immunohistochemical staining for HER2 protein shows strong basolateral membrane staining of the tumor cells

with an immunohistochemistry score of 2+, 21 patients were also detected to have HER2 gene amplification by double-color fluorescent in situ hybridization. Finally, 118 (11.7 %) patients were defined as being positive for tumor HER2 overexpression.

Demographics, tumor-related factors and tumor HER2 overexpression

Correlations between HER2 overexpression and demographic and tumor-related factors are shown in Table 2. HER2 overexpression was more commonly observed in patients of greater age (14.1 %, $p = 0.024$), males (14.3 %, $p < 0.001$), patients with tumors with well-differentiated histology (20.2 %, $p < 0.001$), patients with

Table 2 Correlation of human epidermal growth factor receptor 2 (HER2) overexpression with demographics and tumor-related factors

Variables	HER2 negative, *n* (%)	HER2 positive, *n* (%)	*p* value
Total number	888 (88.3)	118 (11.7)	–
Age (years)			
≤65	477 (90.5)	50 (9.5)	0.024
>65	411 (85.9)	68 (14.1)	
Gender			
Male	580 (85.7)	97 (14.3)	<0.001
Female	308 (93.7)	21 (6.3)	
Tumor location			
Esophagogastric junction	28 (90.4)	3 (9.6)	0.497
Stomach	860 (88.2)	115 (11.8)	
Tumor diameter (cm), median (range)	4.0 (0.4–34.4)	4.9 (1.2–18.0)	0.219
Macroscopic tumor type			
Type 0	461 (88.5)	60 (11.5)	0.002
Type 1, 2	115 (79.3)	30 (20.7)	
Type 3, 4	296 (91.6)	27 (8.4)	
Type 5	16 (94.1)	1 (5.9)	
Grade of differentiation			
Well (tubular, papillary)	395 (79.8)	100 (20.2)	<0.001
Poorly (poorly, signet cell)	472 (96.6)	17 (3.4)	
Others	21 (95.5)	1 (4.5)	
Pathological (p) T[a] disease			
pT1	446 (88.3)	59 (11.7)	0.027
pT2	116 (91.4)	11 (8.6)	
pT3	194 (83.6)	38 (16.4)	
pT4	132 (93.0)	10 (7.0)	
Pathological N[b] disease			
pN0	550 (90.3)	59 (9.7)	0.038
pN1	116 (82.3)	25 (17.7)	
pN2	86 (83.7)	14 (16.3)	
pN3a	73 (79.5)	15 (20.5)	
pN3b	56 (92.9)	4 (7.1)	
Pathological stage			
I	487 (89.5)	57 (10.5)	0.556
II	184 (86.0)	30 (14.0)	
III	164 (87.7)	23 (12.3)	
IV	53 (86.9)	8 (13.1)	
Neo-adjuvant chemotherapy			
Absent	839 (86.7)	113 (13.3)	0.669
Present	49 (90.7)	5 (9.3)	

[a] T, primary tumor

[b] N, regional lymph node

tumors exhibiting expansive growth (macroscopic type 1 or type 2, $p = 0.002$), and patients with lymph node metastasis (N1 disease, 17.7 %; N2 disease, 16.3 %; N3a disease, 20.5 %; $p = 0.038$). Although differences in frequency of HER2 overexpression among the T stages were estimated to be significant ($p = 0.027$), no consistent gradient was observed. The presence of HER2 overexpression in the tumor was not influenced by tumor location, tumor size, pathological stage, or history of administration of neo-adjuvant chemotherapy.

HER2 overexpression and the prognosis after surgery

The 5Y-DSS rates in the patients with and without HER2 overexpression who underwent R0 resection were 87.2 and 87.7 %, respectively. With respect to the 5-year RFS (5Y-RFS) rate after R0 resection, the percentage of patients with and without tumor HER2 overexpression was 81.1 and 83.5 %, respectively. HER2 overexpression in the tumors was not correlated with either DSS (log-rank test; $p = 0.14$) or RFS (log-rank test; $p = 0.718$). Furthermore, tumor HER2 overexpression was also not related to survival in the 58 inoperable patients (data not shown). Univariate analyses using the Cox proportional hazard model (Table 3) to identify the factors influencing DSS and RFS consistently failed to exhibit the prognostic significance of tumor HER2 overexpression. However, age >65 years, male gender, tumor location in the esophagogastric junction, tumor diameter ≥10 cm, tumor exhibiting infiltrative growth (macroscopic type 3 or type 4), T4 disease, and N disease were significant ominous prognostic factors, as previously known. The sites of recurrence in the patients are shown in Table 4. Although patients with tumor HER2 overexpression were predisposed to the development of solitary liver or lymph node metastasis, peritoneal seeding was more frequent in the patients without tumor HER2 overexpression. Surgery was more often performed for operable recurrences in patients with tumor HER2 overexpression, whereas best supportive care tended to be undertaken in patients not showing tumor HER2 overexpression (data not shown).

Discussion

Only R0 resection with prophylactic lymph node dissection has been established as a potentially curative treatment for gastric cancer [13]. Although the beneficial effect of optimal resection based on tumor-related factors would seem to be the maximum achievable for local control and survival, a substantial number of cases eventually show locoregional

Table 3 Univariate analysis by the COX proportional hazard model to identify factors influencing disease-specific survival (DSS) and recurrence-free survival (RFS) in 948 patients who had undergone no residual cancer (R0) resection

Variables	*n*	Risk for cancer-related death			Risk of recurrence		
		HR	95 % CI	Log-rank (*p* value)	HR	95 % CI	Log-rank (*p* value)
Age (years)							
≤65	497	1.000	–	–	1.000	–	–
>65	451	2.003	1.336–3.004	0.001	1.667	1.206–2.304	0.001
Gender							
Female	311	1.000	–	–	1.000	–	–
Male	637	1.542	0.980–2.426	0.059	1.388	0.969–1.988	0.841
Tumor diameter (cm)							
<10	805	1.000	–	–	1.000	–	–
≥10	54	5.016	2.936–8.568	0.004	4.583	3.148–7.480	<0.001
Tumor location							
Stomach	917	1.000	–	–	1.000	–	–
Esophagogastric junction	31	2.774	1.346–5.715	<0.001	3.625	2.090–6.287	<0.001
Macroscopic tumor type							
Type 1 or 2	140	1.000	–	–	1.000	–	–
Type 3 or 4	273	2.037	1.277–3.247	0.002	1.498	1.004–2.235	0.048
Well-differentiated histology	481	1.000	–	–	1.000	–	–
Poorly differentiated histology	450	1.354	0.927–1.979	0.718	1.234	0.910–1.673	0.116
Pathological (p) T[a] disease							
pT1	504	1.000	–	–	1.000	–	–
pT2	126	8.021	3.010–21.37	<0.001	6.891	3.154–15.06	<0.001
pT3	222	19.71	8.406–46.21	<0.001	21.61	11.18–41.77	<0.001
pT4	96	40.63	17.09–96.60	<0.001	33.73	16.93–67.08	<0.001
Pathological (p) N[b] disease							
pN0	605	1.000	–	–	1.000	–	–
pN1	136	3.421	1.676–6.982	0.003	4.532	2.616–7.850	<0.001
pN2	89	8.419	4.451–15.92	<0.001	9.227	5.476–15.55	<0.001
pN3a	79	12.34	6.649–22.91	<0.001	18.03	11.03–29.47	<0.001
pN3b	39	38.02	20.50–70.53	<0.001	31.94	18.32–55.69	<0.001
HER2-negative	836	1.000	–	–	1.000	–	–
HER2-positive	112	0.565	0.262–1.219	0.140	1.109	0.685–1.796	0.718

[a] T, primary tumor

[b] N, regional lymph node

failure. Multimodality therapies are now being assessed, and some clinical trials have already demonstrated the efficacy and safety of adjuvant chemotherapy administered after resection [14–16]. Recently, HER2 expression has attracted attention because of the encouraging results of the ToGA trial. Actually, the results provided the first clue to the potential benefit of tumor biology-based treatment in patients with gastric cancer. Despite the increasing importance of this relationship, the relationship between HER2 overexpression and tumor biology in cases of gastric cancer still remains to be elucidated.

Based on the results of the ToGA trial, several studies have evaluated HER2 overexpression in esophagogastric cancer using validated criteria [17–22]. The frequency of HER2 overexpression has been reported to be 9–16 %, consistent with the results of two major reviews of previous studies [4, 9]. However, the criterion for diagnosing HER2 overexpression still varies among studies. We evaluated HER2-based tumor biology in 1,006 cases of gastric cancer using the following criterion for HER2 overexpression in gastric cancer: immunohistochemistry score of 3+, or immunohistochemistry score of 2+ plus gene amplification; in the present study, using this criterion, the rate of HER2 overexpression was estimated to be 11.7 % in the present study. In a subgroup analysis of the ToGA trial conducted after excluding cases with tumors showing an

Table 4 Sites of recurrence in patients with and without tumor HER2 overexpression

	HER2 negative, *n* (%)	HER2 positive, *n* (%)
Incidence of recurrence	133	19
Site of recurrence, *n* (%)		
Peritoneum	38 (28.6)	1 (5.3)
Liver	23 (17.3)	9 (47.4)
Lymph node	23 (17.3)	5 (26.3)
Lung	3 (2.3)	1 (5.3)
Other sites	16 (12.0)	1 (5.3)
Multiple	26 (19.5)	2 (10.5)
Unclear	4 (3.0)	0 (0.0)

immunohistochemistry score of 0–1+ plus gene amplification, the gain in median survival was 4.2 months in the intent-to-treat population. This gain was superior to the actual gain in the median survival of 2.7 months under the initial entry criteria [10] and was regarded as comparable to the gain of 4.8 months observed in the trial of advanced breast cancer [2, 23]. For obtaining clinical benefit, the criterion for HER2 overexpression of an immunohistochemistry score of 3+ or 2+ plus gene amplification seemed to be the most reliable, and the present study evaluated a large number of patients of gastric cancer using this criterion.

Heterogeneity of HER2 expression level in gastric cancer cases has been suggested as another cause of the discordance of HER2 overexpression among studies [24]. TMA is a useful tool for analyzing a large number of samples, but its major limitation is that tumor cores may not represent the whole tissue section. A recent study that evaluated both whole tissue sections and TMA [19] concluded that use of duplicate TMA samples for each case could minimize the discordance. The concordance rates of immunohistochemistry scores of 2+/3+ and 0/1+ between duplicate TMAs constructed from the same tissue section in a patient were 74.5 and 91.8 %, respectively. Although this was in good agreement with the previously reported results of analysis of biopsy specimens from 261 patients with invasive intestinal-type gastric cancers [25], further investigations are still required to elucidate the reliability of HER2 scoring in TMA samples in gastric cancer.

In the present study, age, gender, growth pattern, grade of differentiation of the tumor, and N disease were found to be correlated with tumor HER2 overexpression (Table 2). Similarly, in the ToGA trial, tumor with HER2 overexpression was reported to be more common in patients with a well-differentiated tumor histology [17–19, 21]. Actually, well-differentiated tumor histology was significantly related to greater age, male gender, and an expansive growth pattern of the tumor in the present study (data not shown). Therefore, age, gender, and the growth pattern of tumors were indirectly related to the likelihood of HER2 overexpression in the tumors. On the other hand, lymph node metastasis was significantly more frequent in patients with poorly differentiated tumor histology (data not shown). Several previous studies have reported the existence of a relationship between the presence of lymph node metastasis and tumor HER2 overexpression [18, 19]. This finding suggests that tumor biology in the subgroup of patients with tumor HER2 overexpression predisposes to spread via the lymphatic system. This finding suggests the potential clinical benefit of HER2-targeted therapy in the adjuvant or neo-adjuvant setting for patients with node-positive gastric cancer. However, no significant correlation between HER2 overexpression and any of the factors of tumor location, depth of tumor (T stage), or pathological stage was observed in the present study.

In the survival analysis, no significant correlation was observed between HER2 overexpression and either DSS or RFS. Actually, recent studies in which the diagnosis of HER2 overexpression was based on immunohistochemical staining using criteria suggested by Hofmann et al. [9] reported conflicting results with respect to the prognostic significance of HER2 overexpression in gastric cancer [20–22]. The inconsistent results could be attributed to multiple factors. First, even the foregoing studies have not standardized treatment of patients having tumors with immunohistochemistry scores of 0 to 2+ plus HER2 gene amplification. As a subgroup analysis of the ToGA trial suggested the indispensable requirement of a different criterion for the diagnosis of HER overexpression in gastric cancer from that in breast cancer, further analysis with the criterion for HER2 overexpression set as an immunohistochemistry scores of 3+ or 2+ plus gene amplification, which is potentially the most reliable at present, would be needed. Second, the studies were of one accord in suggesting that a well-differentiated histology and lymph node metastasis were closely related to HER2 overexpression. However, these two were known as opposing prognostic factors, which could provide controversial results among studies. Gastric cancer with a well-differentiated histology or of the intestinal type generally shows a favorable prognosis, whereas a positive nodal status has an ominous prognostic value. Third, the present study evaluated the relationship between HER2 overexpression and the prognosis in a cohort of operable cases. Fourth, analysis of HER2 overexpression using TMA still remains to be validated, as already stated.

Interestingly, in the analysis of the pattern of recurrence, differences in the sites of recurrence were observed between the two groups with and without tumor HER2 overexpression (Table 4). Solitary metastases in the lymph node or liver were more common in patients with tumor HER2 overexpression, whereas peritoneal seeding and

multiple metastases were more frequent in patients without tumor HER2 overexpression. In a previous analysis of 643 patients administered systemic chemotherapy alone, the 5-year survival rates in the patients bearing metastasis confined to the abdominal lymph nodes and liver were 10.4 and 1.7 %, respectively, which was better than that of 0 % in the patients showing peritoneal dissemination [26]. The slight trend of favorable DSS in patients with HER2 overexpression despite these same patients showing a trend toward poor RFS might be attributable to the higher incidence of recurrence in lymph nodes in these patients. This finding implied that patients with tumor HER2 overexpression possibly included quite a considerable subpopulation responding to chemotherapy and exhibiting a favorable tumor biology. In a subclass analysis of the ToGA trial, lower gain of survival time was observed in an intent-to-treat analysis in the Asian population [10]. Although differences in environmental factors and biology of gastric cancer between Asian and Western populations [27] might be a possible explanation, the large number of patients who received subsequent chemotherapy following failure of first-line chemotherapy could be a likely reason [28]. Survival of patients showing tumor HER2 overexpression in the ToGA trial could have been influenced by the second-line chemotherapies.

Although further elucidation of the HER2-based biology of gastric cancer, which seems to be different from that of breast cancer, is required, multimodality therapy with trastuzumab for operable cases could be limited to a small subgroup. Patients with extensive spread to the lymph nodes may be good candidates. Furthermore, establishment of other treatment targets in patients without tumor HER2 overexpression may be more important to improve overall survival in gastric cancer patients.

In summary, we evaluated the relationship between tumor HER2 overexpression as assessed using a validated immunohistochemistry scoring system and clinical course of the patients in 1,006 cases of gastric cancer. Tumor HER2 overexpression was correlated with a well-differentiated histology and presence of lymph node metastasis. Patients with tumor HER2 overexpression are considered to constitute a subgroup that may be expected to show favorable response to HER2-targeted therapy.

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