Correlation of HER2 Expression with Clinicopathological Characteristics and Prognosis in Resectable Gastric Cancer

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Abstract. *Results from the Trastuzumab for Gastric Cancer (ToGA) trial highlighted the clinical significance of trastuzumab in the treatment of HER2 (Human Epidermal Growth Factor Receptor type 2)-positive gastric cancer. However, whether expression of HER2 is related to prognosis of gastric cancer is still controversial. A total of 360 consecutive patients with gastric cancer who underwent surgical resection in our Department from 1994 to 2007 were analyzed. We performed immunohistochemical analysis of HER2 expression. HER2 expression level was classified into four scores (0, 1+, 2+ and 3+). There were 37 (10%) patients with a score of 3+. A score of 3+ was defined as being HER2-positive. Recurrence-free survival was worse in HER2-positive cases (p=0.045). When the analysis was conducted with intestinal types of cancer, RFS was considerably worse in the HER2-positive group (p=0.011). HER2 expression may have potential as a prognostic factor for intestinal cancer types. Further research is warranted.*

Approximately 100,000 people develop gastric cancer in Japan every year (1). From the result of the SPIRITS trial, treatment with S-1, an oral fluoropyrimidine, and cisplatin have become the standard therapy for advanced gastric cancer in Japan (2). However, new treatments are needed since prognosis remains poor. The human epidermal growth factor receptor 2 (*HER2*) gene, located on chromosome 17q11.2-12, encodes the transmembrane glycoprotein receptor, p185HER2, which is the target for the humanized monoclonal antibody trastuzumab (Herceptin®) (3). *HER2* is

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amplified and overexpressed in approximately 25% of patients with breast cancer, and is associated with an aggressive clinical course and poor prognosis (4). Since 1998, trastuzumab has been used to treat more than 740,000 patients with HER2-positive breast cancer worldwide (5).

The Trastuzumab for Gastric Cancer (ToGA) study was the first randomized, prospective, multicenter, phase III trial to evaluate the efficacy and safety of trastuzumab for the treatment of HER2-positive gastric cancer. The clinically significant overall survival benefit demonstrated that trastuzumab was a new, effective and well-tolerated treatment for HER2-positive gastric cancer (6).

HER2 is now recognized as an important prognostic factor in breast cancer (4). However, the clinicopathological characteristics of HER2-positive gastric cancer are controversial. According to most studies, it was thought that HER2 overexpression was a poor prognostic factor (7-11), but in other studies, HER2 was not associated with gastric cancer outcomes, although these results were published after the release of data from the ToGA study (12-14). Additionally, reports that classified cases according to the clinicopathological characteristics were also seen. In some reports, HER2 was found to be a poor prognostic factor in patients with unresectable gastric cancer (15), as well as in those with liver metastasis of gastric cancer (16).

In addition, it was thought that there were unresolved problems in assessing HER2 amplification/expression in gastric cancer (17, 18). Although in many cases, HER2 overexpression was determined using both immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), it became clear in the ToGA trial that IHC was better than FISH at assessing HER2 overexpression (6).

It is still unclear whether HER2 expression is related to prognosis in gastric cancer. In the present study, we considered clinicopathological factors and HER2 expression in the prognosis of 360 patients with resectable gastric cancer.

Patients and Methods

Patients and specimens. We retrospectively analyzed 360 consecutive patients with any stage of gastric cancer who underwent surgical resection at the Department of Surgery and Science, Kyushu University Hospital, from 1994-2007. The background and follow-up duration of the patients are summarized in Tables I and II. Histological diagnosis was based on World Health Organization criteria (19). Pathological staging was performed according to the tumor-node-metastasis (TNM) classification system revised in 2002 (20).

Immunohistochemistry. The Dako HercepTest, an IHC assay, was performed according to the manufacturer's instructions (21). IHC was performed on formalin-fixed, paraffin-embedded 4 μm-thick tissue sections. Sections were deparaffinized and heat-induced epitope retrieval was performed at 95°C for 40 min. Endogenous peroxidase activity was blocked by treating the sections for 5 min with a peroxidase blocking reagent after which the primary antibody (ready-to-use rabbit polyclonal antibody to human HER2) was applied to the slides at 4°C for 30 min. HER2 detection was carried out using the visualization reagent (dextran-horseradish peroxidase polymer conjugated to goat anti-rabbit immunoglobulins) for 30 min and the Diaminobenzidine chromogen substrate solution for 10 min at room temperature. Slides were lightly counterstained with Dako hematoxylin. Positive and negative controls were included in each assay.
Scoring criteria of immunohistochemical results. Results of IHC HER2 staining were evaluated according to the scoring system of 0, 1+, 2+ and 3+ for gastric cancer (Figure 1) (5, 6). Only a score of 3+ was considered HER2-positive.

Statistical analysis. Relationships among the clinicopathological factors and HER2 expression were analyzed using the χ^2 test and logistic regression analysis. Survival curves were plotted using the Kaplan–Meier method, and significant differences among subgroups were compared using the log-rank test. A *p*-value of less than 0.05 was considered statistically significant.

Results

The study population consisted of 242 men and 118 women (male to female ratio=2:1). Their age ranged from 29 to 90 years. Out of the 360 patients, 163 (45%) had a type of intestinal cancer and 197 (55%) had the other types of gastric cancer. One hundred and sixteen patients had tumor of pT1 and 237 of pT2-4 according to the TNM classification. Available data for each patient are summarized in Tables I and II.

Immunohistochemistry. Overall, 38/360 (10%) patients had tumors that were scored as HER2 3+, 54/360 (15%) were 2+, 86/360 (24%) were 1+ and the remaining 182 tumors (51%) were negative (Figure 1) as determined using IHC.

Association of HER2 with clinicopathological features. We defined only tumors with a score of 3+ as being positive for HER2 expression. There was a significant association between HER2 expression and clinicopathological characteristics. patients with HER2-positive tumors were older (*p*=0.004;

Table I), predominantly males (*p*=0.0035; Table I), and had a higher tumor grade (*p*=0.0005; Table I).

Prognostic significance of HER2 protein expression. Recurrence-free survival (RFS) of patients with HER2-positive tumors was significantly worse than that of patients with HER2-negative tumors (*p*=0.045; Figure 2b), but there was no significant difference in overall survival (OS) (*p*=0.88; Figre 2a).

Analysis focused on intestinal type of cancer. Since HER2 expression was significantly associated with histological differentiation (*p*=0.0007; Table I), we focused on the 163 patients with an intestinal type of cancer. Patients with HER2-positive tumor were mainly males (*p*=0.0036; Table II), and with a more advanced tumor stage (*p*=0.045; Table II). Moreover, the RFS of patients with HER2-positive tumors was worse than that of those with HER2-negative ones (*p*=0.011; Figure 3).

Discussion

HER2 overexpression in a gastric cell line and gastric carcinomas was reported for the first time in 1986 (22, 23). There have been various studies that have assessed HER2 as a potential prognostic factor in patients with gastric cancer, but the role of HER2 as a prognostic factor remains controversial. Some studies reported a direct correlation between HER2 expression and poor survival (12-14). However, other studies have failed to find any association between HER2 expression and prognosis (7-11).

The methods used for IHC analysis of HER2 expression varied according to the study, and this may be one of the important factors giving rise to this observed discrepancy. There exist two main methods used to investigate HER2 expression: protein analysis using IHC, and gene amplification evaluated using FISH. It has been reported that the rate of HER2 protein overexpression varied from 8%-53% and *HER2* gene amplification from 16%-27% in gastric cancer studies (24). The differences among these studies are likely due to several factors, including sample size, study design, differences in geographic location (7) and gastric cancer heterogeneity (25). However, the type of HER2 test carried out and the scoring criteria used had the most impact.

In recent studies, HER2 expression has been defined as being positive when HER2 was scored as 3+ using IHC or when HER2 was FISH-positive and was scored as 2+ using IHC (5, 8, 12, 13, 15, 26). However, this method of scoring is still controversial since the results using IHC and FISH itself show variability. Recently, it was thought that a reasonable prevalence of HER2 positivity in gastric cancer using standard assays and scoring systems was of about 15% (17). In certain reports, only IHC was used because the expression rate was reduced when both IHC and FISH were used (6). From the result of the ToGA trial, it can be seen that IHC

Table I. *Relationship between clinicopathologic factors and HER2 (Human Epidermal Growth Factor Receptor type 2) protein expression.*

Characteristic	All patients (N=360)	HER2 expression		
		Low level (N=322)	High level[1] (N=38)	*p*-Value
Age, years (mean±SD)	63.5±12.0	62.8±12.1	68.7±9.4	0.004
Gender (%)				
Male	242 (67)	209 (65)	33 (87)	0.0035
Female	118 (33)	113 (35)	5 (13)	
Depth of invasion (%)[2]				
Tl	119 (33)	109 (34)	10 (26)	0.35
T2	103 (29)	86 (27)	17 (45)	
T3	110 (30)	103 (32)	7 (18)	
T4	28 (8)	24 (7)	4 (11)	
Tumor grade (%)[3]				
Well, mod	163 (45)	136 (42)	27 (71)	0.0007
pap,por,sig,muc	197 (55)	186 (58)	11 (29)	
Lymph node metastasis (%)				
–	143 (40)	129 (40)	14 (37)	0.70
+	217 (60)	193 (60)	24 (63)	
Stage(%)[3]				
I	160 (44)	143 (45)	17 (45)	0.48
II	58 (16)	54 (17)	4 (10)	
III	64 (18)	56 (17)	8 (21)	
IV	78 (22)	69 (21)	9 (24)	

[1]Cases where more than score 3+ is defined as high level; [2]histological diagnosis was based on World Health Organization criteria (19); [3]pathologic staging was performed according to the tumor-node-metastasis (TNM) classification system revised in 2002 (20).

Table II. *Relationship among clinicopathological factors and HER2 (Human Epidermal Growth Factor Receptor type 2) protein expression in patients with intestinal cancer types.*

Characteristic	All patients (N=163)	HER2 expression		
		Low level (N=136)	High level[1] (N=27)	*p*-Value
Age, years (mean±SD)	67.4±9.51	67.1±0.81	69.3±1.83	0.28
Gender (%)				
Male	117 (72)	92 (68)	25 (93)	0.0036
Female	46 (28)	44 (32)	2 (7)	
Depth of invasion (%)[2]				
T1	77 (47)	68 (50)	9 (33)	0.44
T2	40 (25)	28 (21)	12 (44)	
T3	35 (21)	32 (23)	3 (11)	
T4	11 (7)	8 (6)	3 (11)	
Lymph node metastasis (%)				
–	84 (51)	73 (53)	11 (41)	0.23
+	79 (49)	63 (47)	16 (59)	
Stage (%)[2]				
I	100(61)	88 (65)	12 (45)	0.045
II	17(11)	14 (10)	3 (11)	
III	27 (16)	21 (15)	6 (22)	
IV	19 (12)	13 (10)	6 (22)	

[1]Cases where more than score 3+ is defined as high level; [2]pathological staging was performed according to the tumor-node-metastasis (TNM) classification system revised in 2002 (20).

more accurately reflects HER2 expression than does FISH, therefore, the rate of HER2 positivity is often defined by an HER2 3+ score using IHC in clinical practice (6). In our investigation, we defined only IHC 3+ tumors as being HER2-positive. Therefore, the evaluation of HER2 expression should be carefully considered to obtain reproducible results.

Sub-group analysis sometimes revealed a significant correlation between HER2 expression and prognosis. For example, HER2 overexpression was a significant prognostic factor in patients with gastric cancer with liver metastases (16) or unresectable gastric cancer (15). In our study, a significant prognostic difference was found only in the subgroup of patients with an intestinal type of cancer. It has been suggested that gastric cancer should be divided into subgroups in order to select a more tailored treatment strategy. We found that for RFS but not OS, HER2-positive gastric cancer correlated significantly with poor prognosis. It is possible that HER2-positive gastric cancer might have a poor prognosis, however, this correlation was not found in the case of OS since various types of chemotherapy can be provided in sequence in Japan. Moreover, HER2 seems to be a distinct prognostic factor only in intestinal cancer.

HER2 overexpression plays an important role in the proliferation, apoptosis, and angiogenesis of many solid tumors (27), so it is thought that the prognosis for patients with *HER2* gene amplification should be poor. In breast cancer, HER2 overexpression was shown to be an important clinicopathological prognostic factor. Patients with HER2 overexpression had more metastasis to the lymph nodes and had a more advanced tumor stage (28). A similar result was observed in our study. HER2 overexpression in patients with intestinal types of carcinoma was observed at a more advanced stage and was associated with poor prognosis, so we believe that similarly to breast cancer, *HER2* gene amplification in gastric cancer would result in tumors having both greater invasive and proliferative capacity. From this point of view, perhaps treatment strategies that are used in breast cancer should be applied to patients with HER2-overexpressing gastric cancer.

The importance of taking HER2 positivity into account for the treatment of gastric cancer has been validated (6), therefore, the opportunity of using trastuzumab for the treatment of gastric cancer is increasing. In order to obtain the best outcome for the patient, it will be extremely important to continue to study and optimize the diagnostic methods used in the determination of HER2 expression.
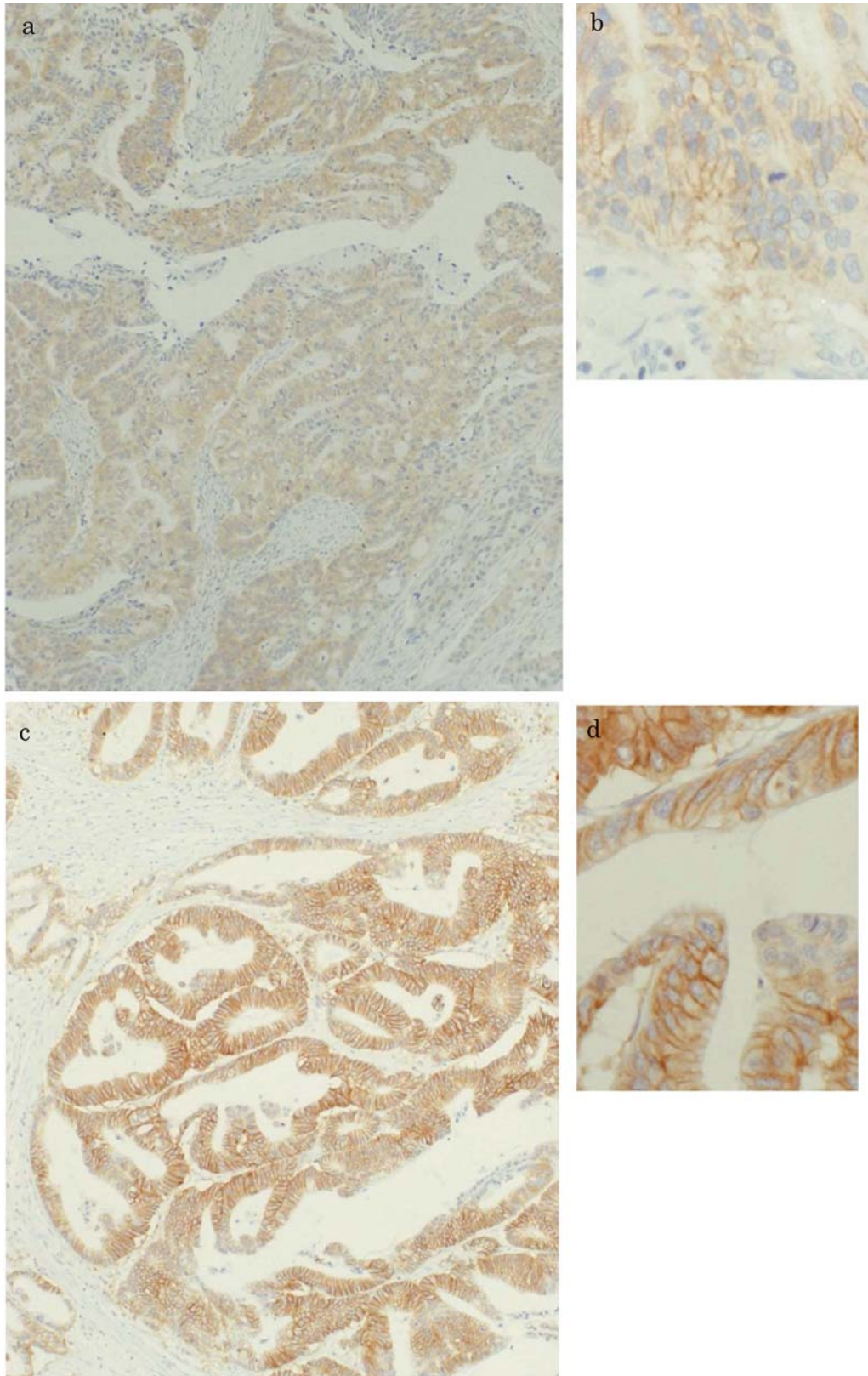
Figure 1. *HER2 (Human Epidermal Growth Factor Receptor type 2) expression. Tumors showing scores of 1+ (a, b) and 3+ (c, d) for HER2 expression. Magnification, a, c: ×100; b, d: ×400. Tumor samples exhibited strong cytoplasmic and nuclear staining for HER2.*
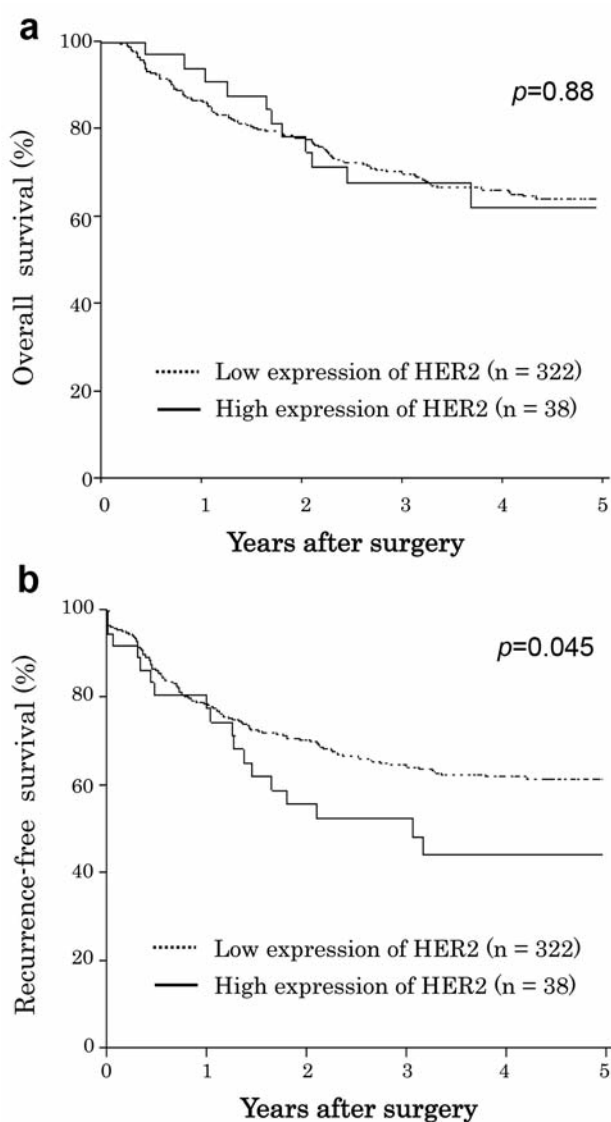
Figure 2. *Kaplan–Meier estimates of 5-year overall survival and 5-year recurrence-free survival rates according to HER2 (Human Epidermal Growth Factor Receptor type 2) expression. High expression of HER2 was significantly associated with poor recurrence-free survival (b) and non-significantly with poor overall survival (a).*
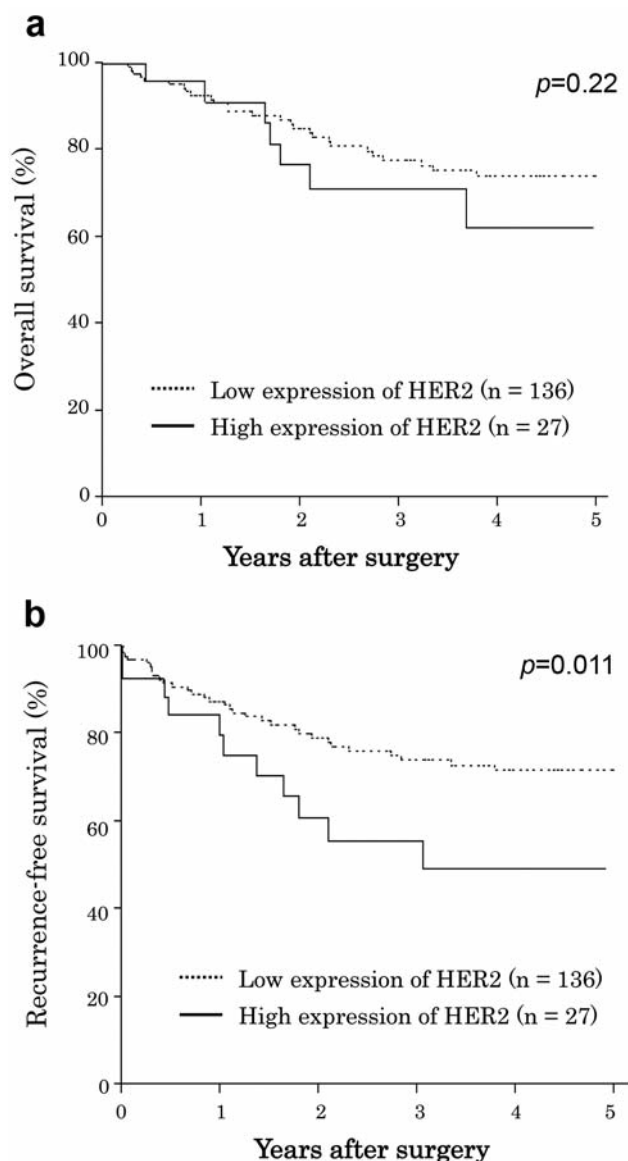

Figure 3. *Kaplan–Meier estimates of 5-year overall survival and 5-year recurrence-free survival rates according to HER2 (Human Epidermal Growth Factor Receptor type 2) expression in patients with intestinal types of gastric cancer. High expression of HER2 was significantly associated with poor recurrence-free survival (b) and non-significantly with poor overall survival (a).*

Acknowledgements

References

1 Foundation for Promotion of Cancer Research. Cancer Statistics in Japan 2013. Available from http://ganjoho.jp/professional/ statistics/ backnumber/2013_jp.html. Last accessed 17 February 2015.

2 Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H and Takeuchi M: 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, 2008.

3 Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC and Norton L: Phase II study of weekly intravenous recombinant humanized anti-p185HER2

monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol *14*: 737-744, 1996.

4 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science *235*: 177-182, 1987.

5 Hofmann M, Stoss O, Shi D, Buttner R, van de Vijver M, Kim W, Ochiai A, Ruschoff J and Henkel T: Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology *52*: 797-805, 2008.

6 Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Ruschoff J, Kang YK and To GATI: Trastuzumab in combination with chemotherapy *versus* chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase III, open-label, randomised controlled trial. Lancet *376*: 687-697, 2010.

7 Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL, da Costa WL, Jr. and Soares FA: Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 are predictors of poor outcome. J Clin Oncol *29*: 3030-3036, 2011.

8 Tanner M, Hollmen M, Junttila TT, Kapanen AI, Tommola S, Soini Y, Helin H, Salo J, Joensuu H, Sihvo E, Elenius K and Isola J: Amplification of HER2 in gastric carcinoma: association with topoisomerase IIα gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. Ann Oncol *16*: 273-278, 2005.

9 Nakajima M, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, Matsuda M, Sakaguchi T, Hirao T and Nakano H: The prognostic significance of amplification and overexpression of c-MET and c-ERBB-2 in human gastric carcinomas. Cancer *85*: 1894-1902, 1999.

10 Park DI, Yun JW, Park JH, Oh SJ, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Yoo CH, Son BH, Cho EY, Chae SW, Kim EJ, Sohn JH, Ryu SH and Sepulveda AR: *HER-2/neu* amplification is an independent prognostic factor in gastric cancer. Dig Dis Sci *51*: 1371-1379, 2006.

11 Yonemura Y, Ninomiya I, Ohoyama S, Kimura H, Yamaguchi A, Fushida S, Kosaka T, Miwa K, Miyazaki I, Endou Y and et al.: Expression of c-erbB-2 oncoprotein in gastric carcinoma. Immunoreactivity for c-ERBB-2 protein is an independent indicator of poor short-term prognosis in patients with gastric carcinoma. Cancer *67*: 2914-2918, 1991.

12 Yano T, Doi T, Ohtsu A, Boku N, Hashizume K, Nakanishi M and Ochiai A: Comparison of *HER2* gene amplification assessed by fluorescence *in situ* hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. Oncol Rep *15*: 65-71, 2006.

13 McCulloch PG, Ochiai A, O'Dowd GM, Nash JR, Sasako M and Hirohashi S: Comparison of the molecular genetics of c-ERB-B2 and p53 expression in stomach cancer in Britain and Japan. Cancer *75*: 920-925, 1995.

14 McCulloch P, Taggart T, Ochiai A, O'Dowd G, Nash J and Sasako M: c-ERBB2 and p53 expression are not associated with stage progression of gastric cancer in Britain or Japan. Eur J Surg Oncol *23*: 304-309, 1997.

15 Gomez-Martin C, Garralda E, Echarri MJ, Ballesteros A, Arcediano A, Rodriguez-Peralto JL, Hidalgo M and Lopez-Rios F: HER2/neu testing for anti-HER2-based therapies in patients with unresectable and/or metastatic gastric cancer. J Clin Pathol *65*: 751-757, 2012.

16 Dang HZ, Yu Y and Jiao SC: Prognosis of HER2 overexpressing gastric cancer patients with liver metastasis. World J Gastroenterol *18*: 2402-2407, 2012.

17 Hsu JT, Chen TC, Tseng JH, Chiu CT, Liu KH, Yeh CN, Hwang TL, Jan YY and Yeh TS: Impact of *HER2* overexpression/ amplification on the prognosis of gastric cancer patients undergoing resection: a single-center study of 1,036 patients. Oncologist *16*: 1706-1713, 2011.

18 Barros-Silva JD, Leitao D, Afonso L, Vieira J, Dinis-Ribeiro M, Fragoso M, Bento MJ, Santos L, Ferreira P, Rego S, Brandao C, Carneiro F, Lopes C, Schmitt F and Teixeira MR: Association of *ERBB2* gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients. Br J Cancer *100*: 487-493, 2009.

19 Jass JR, Sobin LH and Watanabe H: The World Health Organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. Cancer *66*: 2162-2167, 1990.

20 Sobin LH and Wittekind Ch (eds.) TNM Classification of Malignant Tumours. Sixth Edition. New York: Wiley; 2002.

21 Tamura G, Osakabe M, Yanagawa N, Ogata SY, Nomura T, Fukushima N, Ito Y and Tabuchi M: Comparison of HER2 immunohistochemical results using a monoclonal antibody (SV2-61γ) and a polyclonal antibody (for Dako HercepTest) in advanced gastric cancer. Pathol Int *62*: 513-517, 2012.

22 Fukushige S, Matsubara K, Yoshida M, Sasaki M, Suzuki T, Semba K, Toyoshima K and Yamamoto T: Localization of a novel v-erbB-related gene, *c-ERBB-2*, on human chromosome 17 and its amplification in a gastric cancer cell line. Mol Cell Biol *6*: 955-958, 1986.

23 Sakai K, Mori S, Kawamoto T, Taniguchi S, Kobori O, Morioka Y, Kuroki T and Kano K: Expression of epidermal growth factor receptors on normal human gastric epithelia and gastric carcinomas. J Natl Cancer Inst *77*: 1047-1052, 1986.

24 Lorenzen S and Lordick F: How will human epidermal growth factor receptor 2-neu data impact clinical management of gastric cancer? Curr Opin Oncol *23*: 396-402, 2011.

25 Kim MA, Jung EJ, Lee HS, Lee HE, Jeon YK, Yang HK and Kim WH: Evaluation of *HER2* gene status in gastric carcinoma using immunohistochemistry, fluorescence *in situ* hybridization and real-time quantitative polymerase chain reaction. Hum Pathol *38*: 1386-1393, 2007.

26 Kaur A and Dasanu CA: Targeting the HER2 pathway for the therapy of lower esophageal and gastric adenocarcinoma. Expert Opin Pharmacother *12*: 2493-2503, 2011.

27 Tetu B and Brisson J: Prognostic significance of HER-2/neu oncoprotein expression in node-positive breast cancer. The influence of the pattern of immunostaining and adjuvant therapy. Cancer *73*: 2359-2365, 1994.