Esophagus

Mediastinal lymph node metastasis and recurrence in adenocarcinoma of the esophagogastric junction

Yukinori Kurokawa, MD,[a] Naoki Hiki, MD,[b] Takaki Yoshikawa, MD,[c] Kentaro Kishi, MD,[d] Yuichi Ito, MD,[e] Masaki Ohi, MD,[f] Noriko Wada, MD,[a] Shuji Takiguchi, MD,[a] Shinji Mine, MD,[b] Shinichi Hasegawa, MD,[c] Tatsuo Matsuda, MD,[g] *and* **Hiroya Takeuchi, MD,[g]** *Osaka, Tokyo, Yokohama, Nagoya, and Mie, Japan*

Background. *Whether thorough mediastinal dissection is indicated in patients with Siewert type II adenocarcinoma of the esophagogastric junction (EGJ) remains controversial. We conducted a multicenter study to find a preoperative indicator of mediastinal node metastasis.*
Methods. *We retrospectively collected data on 315 patients with pT2-T4 Siewert type II tumors who underwent R0 or R1 resection. The rates of metastasis or recurrence were investigated for the upper, middle, and lower mediastinal lymph nodes. Multivariate logistic analysis was used to identify significant indicators of metastasis or recurrence in the mediastinal nodes.*
Results. *The overall rates of metastasis or recurrence in the upper, middle, and lower mediastinal lymph nodes were 4%, 7%, and 11%, respectively. Rates were significantly higher when the distance from the EGJ to the proximal edge of primary tumor was >3 cm for the upper and middle mediastinal nodes and >2 cm for the lower mediastinal nodes. Multivariate analysis revealed that this distance was the only factor significantly associated with metastasis or recurrence in any mediastinal region. The 5-year overall survival rate in the 12 patients with metastasis in the upper or middle mediastinal lymph nodes was 17%.*
Conclusion. *The distance from the EGJ to the proximal edge of primary tumor may be a significant indicator of metastasis or recurrence in the mediastinal lymph nodes in patients with Siewert type II tumors. Thorough mediastinal lymph node dissection via a transthoracic approach may provide a therapeutic benefit when the distance is >3 cm. (Surgery 2015;157:551-5.)*

From the Department of Gastroenterological Surgery,[a] Osaka University Graduate School of Medicine, Osaka; Department of Gastroenterological Surgery,[b] Gastroenterological Center, Cancer Institute Hospital, Tokyo; Department of Surgery,[c] Kanagawa Cancer Center, Yokohama; Department of Surgery,[d] Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka; Department of Gastroenterological Surgery,[e] Aichi Cancer Center Hospital, Nagoya; Department of Innovative Surgery,[f] Mie University Graduate School of Medicine, Mie; and Department of Surgery,[g] Keio University School of Medicine, Tokyo, Japan

ADENOCARCINOMA OF THE ESOPHAGOGASTRIC JUNCTION (EGJ) is defined as adenocarcinoma with an epicenter within 5 cm of the EGJ and extending into the esophagus.[1] The incidence of EGJ adenocarcinoma has increased substantially in the past few decades.[2-4] EGJ adenocarcinoma is usually classified into 3 categories according to the Siewert system, which is based on the location of the epicenter.[5] Siewert type I tumors are located between 1 and 5 cm above the EGJ and usually arise from an area of intestinal metaplasia in the esophagus. Siewert type II tumors, located 1 cm above to 2 cm below the EGJ, represent true carcinoma of the EGJ that arises from the epithelium of the cardia or short segments of intestinal metaplasia at the EGJ. Siewert type III tumors represent subcardial gastric cancer located 2–5 cm below the EGJ with invasion of the distal esophagus.

Y.K. and N.H. contributed equally to this work.

Conflict of Interest Disclosures: All authors have no conflicts of interest or financial ties to disclose.

Accepted for publication August 7, 2014.

Reprint requests: Takaki Yoshikawa, MD, Kanagawa Cancer Center, Department of Surgery, 1-1-2 Nakao, Asahi-Ku, Yokohama 241-0815, Japan. E-mail: yoshikawat@kcch.jp or Hiroya Takeuchi, MD, Keio University School of Medicine, Department of Surgery, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan. E-mail: htakeuchi@a6.keio.jp.

0039-6060/$ - see front matter

http://dx.doi.org/10.1016/j.surg.2014.08.099

The operative approach for EGJ adenocarcinoma is mainly based on tumor location, that is, the Siewert classification. The transthoracic approach is frequently used for upper (Siewert type I) tumors, whereas the transhiatal approach is used for lower (Siewert type III) tumors. Siewert type II tumors fall between types I and III in most characteristics, so both types of approaches have been attempted for Siewert type II tumors. Although lymph nodes in the lower mediastinal region can be dissected via either approach, the dissection of the upper or middle mediastinal region can be performed only via the transthoracic approach. A Dutch group has conducted a randomized, controlled trial comparing the right transthoracic and transhiatal approaches for Siewert type I and II tumors.[6] Because the survival of patients with Siewert type II tumors was almost the same across both groups, thorough mediastinal dissection via a right thoracotomy could not be recommended for Siewert type II tumors. However, subgroup analysis of patients with limited lymph node metastasis showed that survival in the thoracotomy group was significantly better than survival in the transhiatal group,[7] which suggests that there might be a population with a chance for improved survival with thorough mediastinal dissection via a right thoracotomy. We conducted a multicenter, retrospective study to find a preoperative indicator of mediastinal node metastasis.

METHODS

Patient population. This study included 315 patients with Siewert type II tumors who underwent resection at 7 institutions between March 1986 and October 2010. All tumors were histologically diagnosed as adenocarcinoma. Patients with huge tumors (>10 cm) were excluded from this study. Pathologic T and N stages were based on the International Union Against Cancer tumor–node–metastasis (TNM) staging system.[1] Pathologic T1 tumors or palliative (R2) cases were excluded from this study. The types of surgical approach, esophagectomy, and gastrectomy were not specified in this study. This study (HIK-01) was approved by the Steering Committee of the Young Gastric Surgeons Research Group in Japan and institutional review board of the Osaka University Hospital.

Statistical analysis. The primary endpoint of this study was the rate of metastasis or recurrence in the mediastinal lymph nodes. Mediastinal nodes were classified as belonging to 1 of 3 regions. The upper mediastinal region included upper thoracic paraesophageal, left or right recurrent nerve, pretracheal, and left or right tracheobronchial lymph nodes. The middle mediastinal region included subcarinal, middle thoracic paraesophageal, and left or right main bronchus lymph nodes. The lower mediastinal region included lower thoracic paraesophageal, supradiaphragmatic, and posterior mediastinal lymph nodes.

Recurrence-free survival was defined as the time from surgery to either the first recurrence or death from any cause. Survival curves were estimated using the Kaplan–Meier method. The impact of clinicopathologic factors on lymph node metastasis or recurrence was investigated using multivariate logistic regression analysis. All statistical analyses were performed using SPSS Statistics software, version 20 (IBM Corp, Armonk, NY).

RESULTS

The clinicopathologic characteristics of the 315 patients are shown in Table I. The median tumor size was 5.5 cm and the median distance from the EGJ to the proximal edge of primary tumor was 1.5 cm. Right thoracotomy was performed in 30% of patients. Pathologically positive lymph nodes were shown in 76% of patients.

Of the 315 patients, there were 18 (6%), 33 (11%), and 176 (56%) patients who underwent upper, middle, and lower mediastinal regional lymph node dissection, respectively. Metastasis rates among the dissected cases were >16% in any mediastinal lymph node region, and recurrence rates among the undissected cases were <4% in any region (Table II). The overall rates of metastasis or recurrence in the upper, middle, and lower mediastinal lymph nodes were 4%, 7%, and 11%, respectively. Metastasis or recurrence rates were evaluated after dividing patients into 4 subgroups according to the distance from the EGJ to the proximal edge of primary tumor (Table III). The rate of metastasis or recurrence in the upper or middle mediastinal nodes was significantly higher when the distance was >3 cm, and >2 cm for the lower mediastinal nodes.

Multivariate logistic regression analysis was conducted using 4 factors (histologic type, tumor size, pT stage, and distance from the EGJ to the proximal edge of primary tumor) for each of the 3 regions. Only the distance from the EGJ to the proximal edge of primary tumor was significantly associated with metastasis or recurrence in any mediastinal lymph node region (upper mediastinal nodes, $P = .009$; middle mediastinal nodes, $P = .006$; lower mediastinal nodes, $P = .008$; Table IV). When multivariate analysis was conducted using 5 factors, including preoperative chemotherapy, the significant results for the distance from the EGJ to the proximal edge were essentially unchanged (upper

Table I. Clinicopathologic characteristics ($n = 315$)

Characteristic	n *(%) or median (range)*
Age (y)	63 (18–88)
Sex	
Male	248 (78.7)
Female	67 (21.3)
Histologic type	
Differentiated	198 (62.9)
Undifferentiated	112 (35.6)
Unknown	5 (1.6)
Tumor size (cm)	5.5 (0.8–10.0)
Distance from the EGJ to the proximal edge of primary tumor (cm)	1.5 (0–5.5)
Surgical approach	
Transhiatal	192 (61.0)
Right thoracotomy	93 (29.5)
Left thoracotomy	30 (9.5)
Type of esophagectomy	
Lower	293 (93.0)
Subtotal	22 (7.0)
Type of gastrectomy	
Total	243 (77.1)
Proximal	72 (22.9)
Pathologic T stage	
T2	57 (18.1)
T3	142 (45.1)
T4	116 (36.8)
Pathologic N stage	
N0	75 (23.8)
N1	68 (21.6)
N2	87 (27.6)
N3	85 (27.0)
Preoperative chemotherapy	
No	271 (86.0)
Yes	44 (14.0)

T and N stages are based on the International Union Against Cancer tumor–node–metastasis (TNM) classification, 7th edition.
EGJ, Esophagogastric junction.

mediastinal nodes, $P = .009$; middle mediastinal nodes, $P = .005$; lower mediastinal nodes, $P = .010$).

After a median follow-up of 60 months for censored patients, the 5-year overall and recurrence-free survival rates of all 315 patients were 51% and 43%, respectively (Figure). The 5-year overall survival rate of the 12 patients with metastasis in the upper or middle mediastinal lymph nodes was 17%, compared with 23% for the 31 patients with metastasis in the lower mediastinal lymph nodes.

DISCUSSION

This study demonstrates that the metastasis or recurrence rate in the mediastinal nodes varies remarkably according to the distance from the EGJ to the proximal edge of primary tumor. Patients with a distance of >3 cm had a much greater probability of metastasis or recurrence in the upper or middle mediastinal nodes. We cannot dissect the lymph nodes in these regions via the transhiatal approach, although the lymph nodes in the lower mediastinal region can be dissected partially. The 5-year overall survival rate in the patients with metastasis in the upper or middle mediastinal lymph nodes was around 20%. Thus, a right transthoracic approach may be better when the distance from the EGJ to the proximal edge of primary tumor is >3 cm, not only for ensuring an adequate proximal margin, but also for thorough mediastinal lymph node dissection.

A Dutch trial comparing the right transthoracic and transhiatal approaches in patients with Siewert type I and II tumors found no difference in survival between the 2 approaches.[6] Subgroup analysis showed that thorough mediastinal dissection via a right thoracotomy significantly improved survival in patients with metastasis in 1–8 lymph nodes. However, because the number of metastatic lymph nodes cannot be evaluated preoperatively, we have to select either the right transthoracic or transhiatal approach without adequate information, balancing the morbidity of a right thoracotomy against the potential survival benefit. In our study, 17 of the 36 patients (47%) with tumors >3 cm from the EGJ to its proximal edge of primary tumor had metastases in 1–8 lymph nodes. Thus, these 2 subgroups would share many of the same patients. Because we can know the distance from the EGJ to the proximal edge of primary tumor preoperatively by esophagogastroduodenoscopy or upper gastrointestinal series, it would be a more useful indication for thorough mediastinal dissection via a right thoracotomy to obtain therapeutic benefit.

A Japanese group conducted another randomized, controlled trial comparing the left transthoracic and transhiatal approaches in patients with mainly Siewert type II and III tumors.[8] Although the left transthoracic approach enabled complete dissection of the lower mediastinal nodes, this study demonstrated that there was no survival benefit and higher morbidity associated with the transthoracic approach. However, this Japanese randomized, controlled trial excluded patients if the distance from the EGJ to the proximal edge of primary tumor was >3 cm. The present study indicates that the rate of metastasis or recurrence in the lower mediastinal nodes was remarkably higher when the distance is >2 cm, which might be 1 reason why the previous Japanese randomized, control trial failed to demonstrate a survival benefit with the left transthoracic

Table II. Metastasis or recurrence rates in the upper, middle, and lower mediastinal lymph nodes

Location of mediastinal lymph nodes	*Metastasis rate among patients who underwent dissection, % (n/N)*	*Recurrence rate among patients who did not undergo dissection, % (n/N)*	*Overall metastasis or recurrence rate, % (n/N)*
Upper	16.7 (3/18)	3.0 (9/297)	3.8 (12/315)
Middle	33.3 (11/33)	3.9 (11/282)	7.0 (22/315)
Lower	17.6 (31/176)	3.6 (5/139)	11.4 (36/315)

Table III. Metastasis or recurrence rates in the upper, middle, and lower mediastinal lymph nodes stratified by the distance from the EGJ to the proximal edge of primary tumor

	Distance from the EGJ to the proximal edge of primary tumor (cm), % (n/N)			
Location of mediastinal nodes	*0–1.0*	*1.1–2.0*	*2.1–3.0*	*>3.0*
Upper	0.9 (1/115)	1.1 (1/90)	6.8 (5/74)	13.9 (5/36)
Middle	2.6 (3/115)	5.6 (5/90)	9.5 (7/74)	19.4 (7/36)
Lower	1.7 (2/115)	5.6 (5/90)	24.3 (18/74)	30.6 (11/36)

EGJ, Esophagogastric junction.

Table IV. Multivariate analysis of metastasis or recurrence in the upper, middle, and lower mediastinal lymph nodes

	Upper mediastinal nodes			*Middle mediastinal nodes*			*Lower mediastinal nodes*		
Variable	*OR*	*95% CI*	P *value*	*OR*	*95% CI*	P *value*	*OR*	*95% CI*	P *value*
Histology undifferentiated	2.98	0.81–10.90	.099	1.54	0.60–3.93	.37	0.71	0.32–1.59	.41
Tumor size > 5.0 cm	2.25	0.40–12.49	.36	0.75	0.27–2.13	.59	1.47	0.63–3.42	.37
Pathologic T stage T3–4	0.96	0.11–8.61	.97	3.70	0.46–29.47	.22	2.97	0.66–13.35	.16
Distance from the EGJ to the proximal edge of primary tumor > 3.0 cm	6.74	1.63–27.95	.009	4.82	1.56–14.92	.006	3.30	1.36–8.02	.008

T stage is based on the International Union Against Cancer tumor–node–metastasis (TNM) classification, 7th edition.
EGJ, Esophagogastric junction.

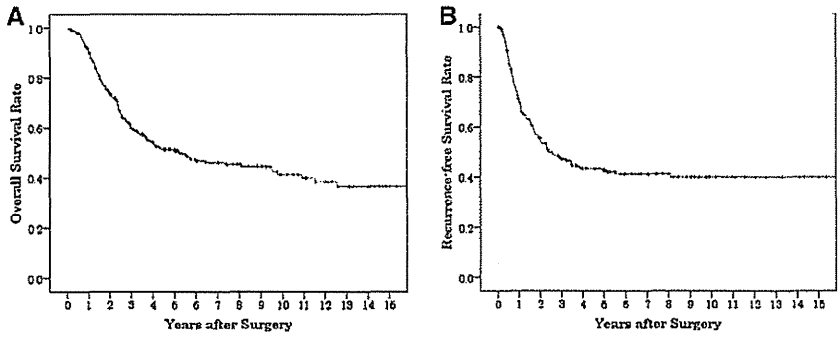

Figure. Overall survival curves (*A*) and recurrence-free survival curves (*B*) in patients with Siewert type II tumors.

approach, even in the subgroup of patients with Siewert type II tumors.

In our study, more than one half of the patients underwent lymph node dissection in the lower mediastinal region, and the metastasis rate in the lower mediastinal nodes was 18%. This rate was similar to that in previous studies.[9-13] On the other hand, because only 5–10% of patients underwent lymph node dissection in the upper and middle mediastinal regions, selection bias may be an issue.

Instead, we investigated the recurrence rate among patients who did not undergo dissection, with a long follow-up period. However, recurrence does not always reflect metastasis at the time of surgery, particularly in patients with recurrence in the systemic lymph nodes. A nationwide, large-scale, prospective study to more accurately determine the rate of metastasis to the mediastinal nodes in EGJ cancer is now ongoing in Japan.

Another limitation of this study was the lack of treatment allocation. The choice of operative methods (transhiatal or thoracotomy; undergo mediastinal lymph node dissection or not) depended on the preference of each participating institution. The low rate (<4%) of mediastinal lymph node recurrence among the undissected cases was probably owing to appropriate selection by surgeon not to undergo mediastinal lymph node dissection. Indeed, the proportion of patients with tumors >3 cm from the EGJ to the proximal tumor edge among 33 dissected cases accounted for one third, whereas that among 282 undissected cases was only 9% ($P < .001$). Surgeon judgment, therefore, in this study was reliable in predicting mediastinal recurrence. Preoperative chemotherapy was also accomplished by surgeon preference in this study. Although preoperative chemotherapy has been reported to contribute to tumor downstaging and improved survival,[14-16] only 14% of patients received preoperative chemotherapy in this study. We also confirmed no significant effect of preoperative chemotherapy on the metastasis or recurrence rate by multivariate analysis.

To our knowledge, this is the first study to evaluate the relationship between the distance from the EGJ to the proximal tumor edge and the metastasis or recurrence rate in the mediastinal lymph nodes. We recommend preoperative evaluation of the distance from the EGJ to the proximal tumor edge to consider the indication of thorough mediastinal dissection. A randomized, controlled trial is warranted to evaluate the efficacy of thorough mediastinal dissection for this patient population.

REFERENCES

1. Sobin LH, Gospodarowicz MK, Wittekind CH. TNM classification of Malignant Tumours. 7th ed. New York: Wiley-Blackwell; 2009.
2. Devesa SS, Blot WJ, Fraumeni JF Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83:2049-53.
3. Bollschweiler E, Wolfgarten E, Gutschow C, Hölscher AH. Demographic variations in the rising incidence of esophageal adenocarcinoma in white males. Cancer 2001;92: 549-55.
4. Crew KD, Neugut AI. Epidemiology of upper gastrointestinal malignancies. Semin Oncol 2004;31:450-64.
5. Siewert JR, Stein HJ. Classification of adenocarcinoma of the oesophagogastric junction. Br J Surg 1998;85:1457-9.
6. Hulscher JB, van Sandick JW, de Boer AG, Wijnhoven BP, Tijssen JG, Fockens P, et al. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the esophagus. N Engl J Med 2002; 347:1662-9.
7. Omloo JM, Lagarde SM, Hulscher JB, Reitsma JB, Fockens P, van Dekken H, et al. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the mid/distal esophagus: five-year survival of a randomized clinical trial. Ann Surg 2007;246:992-1000.
8. Sasako M, Sano T, Yamamoto S, Sairenji M, Arai K, Kinoshita T, et al. Left thoracoabdominal approach versus abdominal-transhiatal approach for gastric cancer of the cardia or subcarsia: a randomised controlled trial. Lancet Oncol 2006;7:644-51.
9. Siewert JR, Feith M, Werner M, Stein HJ. Adenocarcinoma of the esophagogastric junction: results of surgical therapy based on anatomical/topographic classification in 1,002 consecutive patients. Ann Surg 2000;232:353-61.
10. Nunobe S, Ohyama S, Sonoo H, Hiki N, Fukunaga T, Seto Y, et al. Benefit of mediastinal and para-aortic lymph-node dissection for advanced gastric cancer with esophageal invasion. J Surg Oncol 2008;97:392-5.
11. Matsumoto S, Takayama T, Wakatsuki K, Enomoto K, Tanaka T, Migita K, et al. Surgical outcomes for cancer at the gastroesophageal junction. Am Surg 2012;78:1285-91.
12. Mine S, Sano T, Hiki K, Yamada K, Nunobe S, Yamaguchi T. Lymphadectomy around the left renal vein in Siewert type II adenocarcinoma of the oesophagogastric junction. Br J Surg 2013;100:261-6.
13. Hasegawa S, Yoshikawa T, Rino Y, Oshima T, Aoyama T, Hayashi T, et al. Priority of lymph node dissection for Siewert type II/III adenocarcinoma of the esophagogastric Junction. Ann Surg Oncol 2013;20:4252-9.
14. Medical Research Council Oesophageal Cancer Working Group. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet 2002;359:1727-33.
15. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006;355:11-20.
16. Grbski V, Burmeister B, Smithers BM, Foo K, Zalcberg J, Simes J, Australasian Gastro-Intestinal Trials Group. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol 2007;8:226-34.

Gastric Cancer
DOI 10.1007/s10120-014-0430-7

ORIGINAL ARTICLE

Multicenter large-scale study of prognostic impact of HER2 expression in patients with resectable gastric cancer

Yukinori Kurokawa · Nariaki Matsuura · Yutaka Kimura · Shinichi Adachi · Junya Fujita · Hiroshi Imamura · Kenji Kobayashi · Yuhki Yokoyama · Mohammed Nouri Shaker · Shuji Takiguchi · Masaki Mori · Yuichiro Doki

Received: 11 June 2014 / Accepted: 30 August 2014

Abstract
Background Although some small-scale studies have suggested that human epidermal growth factor receptor 2 (HER2)-positive status in gastric cancer is associated with poor outcomes, the prognostic value of HER2 is still controversial. Since intratumoral HER2 heterogeneity is also an important issue, a multicenter large-scale study was conducted to evaluate the prognostic impacts of HER2 expression and intratumoral heterogeneity in gastric cancer.
Methods This study included 1,148 gastric cancer patients who underwent gastrectomy in 11 institutions. HER2 expression was centrally evaluated with immunohistochemistry and fluorescence in situ hybridization, and intratumoral HER2 heterogeneity was evaluated for HER2-positive tumors. Overall survival was compared between HER2-positive and HER2-negative patients and between the homogeneous and heterogeneous groups.
Results The HER2-positive rate was 15.7 %, and HER2 expression was significantly associated with histological type. HER2 expression scores obtained by immunohistochemistry showed a distinct influence on survival, and HER2-positive patients showed much poorer survival than HER2-negative patients [hazard ratio (HR) 1.59, 95 % confidence interval (CI) 1.24–2.02; $P < 0.001$). The subgroup analysis by pathological tumor stage showed a similar trend of poor survival in HER2-positive patients. Both intestinal type and diffuse type showed significant poor survival in HER2-positive patients. Cox multivariate analysis revealed that HER2 expression was an independent prognostic factor (HR 1.96, 95 % CI 1.51–2.55; $P < 0.001$). HER2 heterogeneity was observed in 75.4 % of HER2-positive cases, but the prognosis in the heterogeneous group was similar to that in the homogeneous group.
Conclusions Our study demonstrated that HER2 overexpression is an independent prognostic factor in patients with any stage of resectable gastric cancer. Intratumoral HER2 heterogeneity did not affect prognosis.

Keywords Human epidermal growth factor receptor 2 · Epidermal growth factor receptor 2 · *ERBB2* · Trastuzumab · Heterogeneity

Y. Kurokawa (✉) · S. Takiguchi · M. Mori · Y. Doki
Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2-E2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: ykurokawa@gesurg.med.osaka-u.ac.jp

N. Matsuura · Y. Yokoyama · M. N. Shaker
Department of Molecular Pathology, Osaka University Graduate School of Medicine and Health Science, Osaka, Japan

Y. Kimura
Department of Surgery, Sakai Municipal Hospital, Osaka, Japan

S. Adachi
Department of Surgery, Ikeda City Hospital, Osaka, Japan

J. Fujita
Department of Surgery, NTT West Osaka Hospital, Osaka, Japan

H. Imamura
Department of Surgery, Toyonaka Municipal Hospital, Osaka, Japan

K. Kobayashi
Department of Surgery, Kinki Central Hospital, Itami, Hyogo, Japan

Introduction

Gastric cancer remains a major health issue and a frequent cause of cancer death worldwide, although the prevalence

Published online: 16 September 2014

and mortality of the disease have gradually decreased [1]. In eastern Asia, including Japan, the incidence of gastric cancer is still high despite advances in treatment and subsequent improvements in prognosis. Complete resection of localized tumors is the primary treatment, but recurrence and metastatic spread occur frequently nevertheless. The development of new agents and combination chemotherapies for advanced gastric cancer has led to a steady increase in overall survival (OS). Recently, targeted therapies have significantly impacted the treatment strategy for many common malignancies, including breast, colorectal, and lung cancers. Among these targeted therapies, monoclonal antibody against human epidermal growth factor receptor 2 (HER2) was demonstrated to be highly effective in HER2-positive breast cancer, and was approved by the US Food and Drug Administration as the first molecularly targeted agent for solid cancer [2]. In breast cancer, it is well known that HER2 overexpression is a prognostic factor and also a predictive factor for treatment with trastuzumab (anti-human HER2 monoclonal antibody) [3, 4]. In addition, a randomized controlled trial (ToGA study) recently demonstrated that treatment with trastuzumab could extend the OS of patients with HER2-positive gastric cancer [5].

Regarding the relationship between HER2 status and prognosis, some studies have suggested that HER2-positive status in gastric cancer is associated with poor outcomes and aggressive disease [6–8]. However, the sample sizes of these studies were relatively small, and not all studies have shown an association with poor outcomes [9, 10], so the prognostic value of HER2 in gastric cancer is still controversial. We therefore conducted a multicenter large-scale study to clarify the prognostic impact of HER2 expression in patients with gastric cancer. Furthermore, since intratumoral HER2 heterogeneity is an important issue, especially in gastric cancer [11], we evaluated the clinicopathological characteristics of heterogeneous cases and their impact on prognosis.

Methods

Patient population

This study included patients with gastric cancer of any stage who underwent gastrectomy between 2000 and 2006 at any of the 11 institutions of the Osaka University Clinical Research Group for Gastroenterological Surgery. Patients who received chemotherapy or radiation therapy before surgery were excluded. No patients were given trastuzumab, even after recurrence. All tumors were histologically diagnosed as adenocarcinoma of the stomach. Pathological tumor staging was performed according to the seventh edition of the International Union Against Cancer TNM classification [12]. This study was approved by the institutional review board of each institution involved.

Immunohistochemistry

Archived formalin-fixed, paraffin-embedded specimens were shipped to the institution performing central pathology review. Each specimen was composed of several blocks of tumor oriented longitudinally from proximal to distal stomach, including adjacent normal gastric tissue in each case. Immunohistochemical analysis was carried out on 4-μm-thick tissue sections. The primary antibody was mouse monoclonal anti-HER2 (clone 4B5, Ventana Medical Systems, Tucson, AZ, USA). Immunostaining was performed using a Ventana BenchMark XT® autoimmunostainer with an iVIEW DAB universal kit (Ventana Medical Systems, Tucson, AZ, USA). Antigen retrieval was performed by autoclaving in EDTA-base buffer, pH 8.5 at 98 °C for 60 min. After endogenous peroxidase had been briefly blocked, the sections were incubated with primary antibodies at 37 °C for 32 min, then incubated with a mixture of goat biotinylated anti-mouse IgG, anti-mouse IgM, and anti-rabbit IgG antibodies for 8 min and with peroxidase-conjugated streptavidin for 8 min. Visualization was performed by incubation in 3-diaminobenzidine solution for 8 min, followed by signal amplification with copper sulfate for 4 min and counterstaining with hematoxylin for 4 min. Specimens of HER2-positive breast cancer were used as a positive control, and slides processed without the primary antibodies were used as negative controls.

Evaluations

Central pathologists independently performed immunohistochemical analysis without prior knowledge of the patients' clinical data. Immunostaining of the cell membranes of HER2-positive tumors was scored using a four-grade scale (0/ 1+/ 2+/ 3+) (ToGA score): 0, no reactivity or membranous reactivity in less than 10 % of tumor cells; 1+, faint or barely perceptible membranous reactivity in at least 10 % of tumor cells; 2+, weak to moderate complete, basolateral or lateral membranous reactivity in at least 10 % of tumor cells; and 3+, strong complete, basolateral or lateral membranous reactivity in at least 10 % of tumor cells. For specimens with an immunohistochemistry (IHC) score of 2+ only, fluorescence in situ hybridization (FISH) analysis of HER2 status was performed with a Path Vysion HER2 DNA probe kit (Vysis/Abbott, Abbott Park, IL, USA) following the manufacturer's instructions. For FISH, the total numbers of HER2 and chromosome 17 signals were counted in at least 20 tumor cell nuclei in two different areas. The HER2/chromosome 17 ratios were

interpreted in accordance with the ToGA FISH scoring scheme for HER2 testing in gastric and gastroesophageal junction cancer as follows: less than 2.0, HER2 gene not amplified; 2.0 or more, HER2 gene amplified. An IHC score of 3+ or an IHC score of 2+ with FISH positivity was defined as HER2 positive, whereas IHC scores of 0 or 1+, or an IHC score of 2+ with FISH negativity was defined as HER2 negative [5, 11]. Regarding intratumoral heterogeneity of HER2 overexpression, cases with HER2 IHC scores of 2+ or 3+ in more than 90 % of tumor cells were considered to be homogeneous, whereas heterogeneous cases were defined as those in which 10–90 % of tumor cells had IHC scores of 2+ or 3+.

Statistical analysis

The relationship between HER2 expression and clinicopathological factors was analyzed using the chi square test for categorical variables and the Mann–Whitney *U* test for continuous variables. OS was defined as the interval from the date of surgery to the date of death from any cause. Survival rates were estimated using the Kaplan–Meier method and were compared with the log-rank test. The hazard ratio (HR) for death in HER2-positive patients was estimated with a Cox proportional hazard model. Multivariate Cox regression analysis was performed to adjust for potential confounding factors. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS Statistics, version 20 (IBM, Armonk, NY, USA).

Results

Expression status

The IHC results for HER2 expression in 1,148 tumors were as follows: score 0, 657 (57.2 %); score 1+, 207 (18.0 %); score 2+, 123 (10.7 %); and score 3+, 161 (14.0 %). Of the 123 tumors with an IHC score of 2+, 19 (15.4 %) were HER2 positive by FISH. In total, 180 (15.7 %) of all 1,148 tumors were diagnosed as HER2 positive. The patients' clinicopathological factors were compared by HER2 status (Table 1). HER2-positive cases were found more frequently in the intestinal type of adenocarcinoma ($P < 0.001$). Tumors located in the upper body of the stomach were more likely to be HER2 positive. The HER2 positivity rates according to the pathological tumor staging were 16.4 % in stage I tumors, 11.8 % in stage II tumors, 13.3 % in stage III tumors, and 24.3 % in stage IV tumors, respectively. The other factors, including pT and pN stages, showed no correlation with HER2 positivity.

Table 1 Characteristics of 1,148 patients

Characteristics	HER2 positive ($n = 180$)	HER2 negative ($n = 968$)	*P*
Age (years)			
Median	67.5	67	0.17
Range	37–87	31–98	
Sex			
Male	133	657	0.11
Female	47	311	
Location			
Upper	49	206	0.078
Middle/ lower	131	762	
Histological type			
Intestinal type	142	478	<0.001
Diffuse type	38	490	
pT			
T1	76	369	0.36
T2	24	106	
T3	32	220	
T4	48	273	
pN			
N0	90	517	0.21
N1	22	154	
N2	28	134	
N3	40	163	
Pathological stage			
I	79	403	0.006
II	30	225	
III	35	228	
IV	36	112	

HER2 human epidermal growth factor receptor 2

Survival rates

After the median follow-up period of 62 months, the OS in 1,148 patients was analyzed to evaluate the prognostic impact of HER2 expression. HER2 expression scores obtained by IHC showed a distinct influence on survival, although patients with HER2 expression scores of both 1+ and 2+ had similar survival curves (Fig. 1a). When we compared OS in terms of HER2 status, patients with HER2-positive gastric cancer showed much poorer survival than those whose tumors were HER2 negative [HR 1.59, 95 % confidence interval (CI) 1.24–2.02; log-rank $P < 0.001$] (Fig. 1b).

Subgroup analysis by pathological tumor stage showed a similar trend of poor survival in HER2-positive patients, although a higher HR for death was obtained in earlier stages, as follows: stage I, HR 2.04 (95 % CI 1.14–3.65),

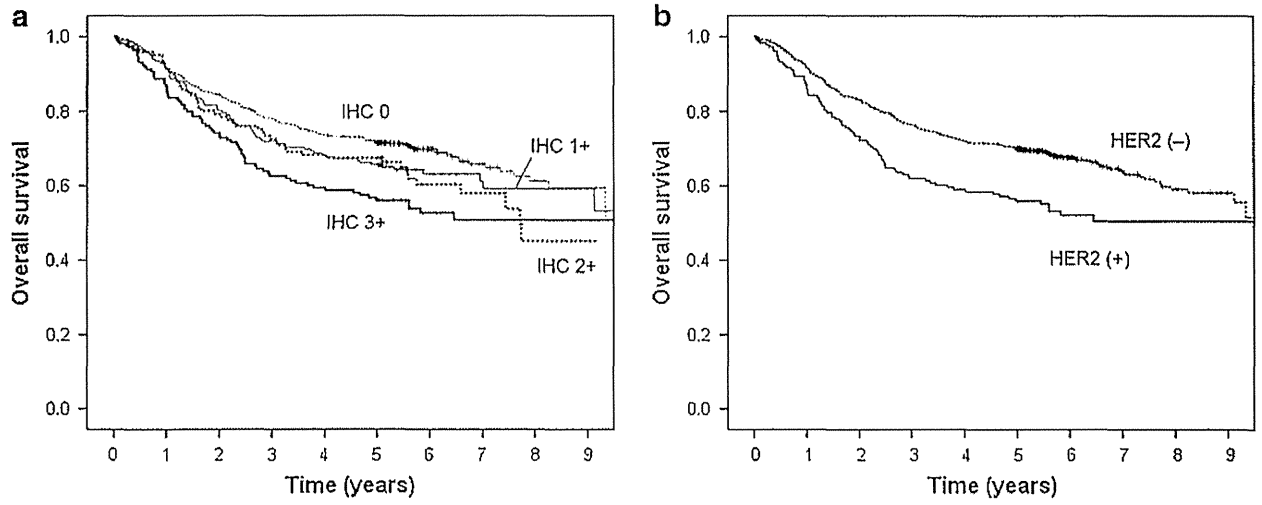

Fig. 1 Kaplan–Meier overall survival for all 1,148 patients according to a human epidermal growth factor receptor 2 (*HER2*) immunohistochemistry (*IHC*) scores, and b HER2 overexpression status

$P = 0.015$ (Fig. 2a); stage II, HR 1.89 (95 % CI 1.02–3.53), $P = 0.041$ (Fig. 2b); stage III, HR 1.47 (95 % CI 0.95–2.28), $P = 0.082$ (Fig. 2c); and stage IV, HR 1.43 (95 % CI 0.95–2.16), $P = 0.084$ (Fig. 2d). Regarding the histological type, both intestinal type and diffuse type showed significantly poor survival in HER2-positive patients [intestinal type, HR 1.70 (95 % CI 1.26–2.30), $P < 0.001$; diffuse type, HR 1.88 (95 % CI 1.20–2.97), $P = 0.005$]. HER2 positivity was also associated with significantly poorer survival both in patients who received adjuvant treatment and in those who did not [with adjuvant, HR 1.94 (95 % CI 1.34–2.80), $P < 0.001$; without adjuvant, HR 1.46 (95 % CI 1.05–2.04), $P = 0.023$].

Cox multivariate analysis of HER2 status and seven clinicopathological factors (age, sex, location, histological type, pT, pN, adjuvant chemotherapy) revealed that age, pT stage, pN stage, and HER2 status were independent significant prognostic factors (Table 2). The adjusted HR for death in HER2-positive patients was 1.96 (95 % CI 1.51–2.55).

HER2 heterogeneity

Intratumoral heterogeneity of HER2 overexpression by IHC was evaluated for HER2-positive tumors. Of the 175 tumors that could be evaluated for heterogeneity, 132 (75.4 %) showed heterogeneous HER2 overexpression (Fig. 3). There were no homogeneous cases among the 18 tumors that had IHC scores of 2+. Heterogeneous cases were significantly more likely to be classified as the diffuse type (Table 3). The distribution of pT stage also differed significantly between the homogeneous and heterogeneous groups. The OS in the heterogeneous group was similar to that in the homogeneous group (HR 0.88, 95 % CI 0.54–1.45; $P = 0.63$) (Fig. 4).

Discussion

Our multicenter large-scale study of 1,148 patients demonstrated that HER2 overexpression was a significant factor associated with poor prognosis in patients with resectable gastric cancer. Differences in HER2 expression scores (0/1+, 2+/3+) obtained by IHC showed a distinct influence on OS. HER2-positive patients with cancer of all stages had shorter survival than HER2-negative patients.

Although the prognostic impact of HER2 expression in patients with resectable gastric cancer has been previously examined in many small-scale retrospective studies, the prognostic value of HER2 is still controversial. A recent systematic review which included only studies with over 100 patients reported that most of the publications (71 %) showed that a HER2-positive status was associated with poor survival and/or clinicopathological characteristics, such as serosal invasion, lymph node metastases, or distant metastases [13]. Kim et al. [8] performed a tissue microarray analysis of HER2 expression in 595 Korean gastric cancer patients and found that HER2 overexpression was an independent prognostic factor in differentiated, resectable gastric cancer. On the other hand, Terashima et al. [10] reported no survival impact of HER2 expression in 829 Japanese gastric cancer patients. Several Japanese investigators might have considered this as a final answer to the long-debated issue, since the study analyzed a large number of archival specimens obtained in collaboration with the pivotal nationwide phase III trial. Curiously, the

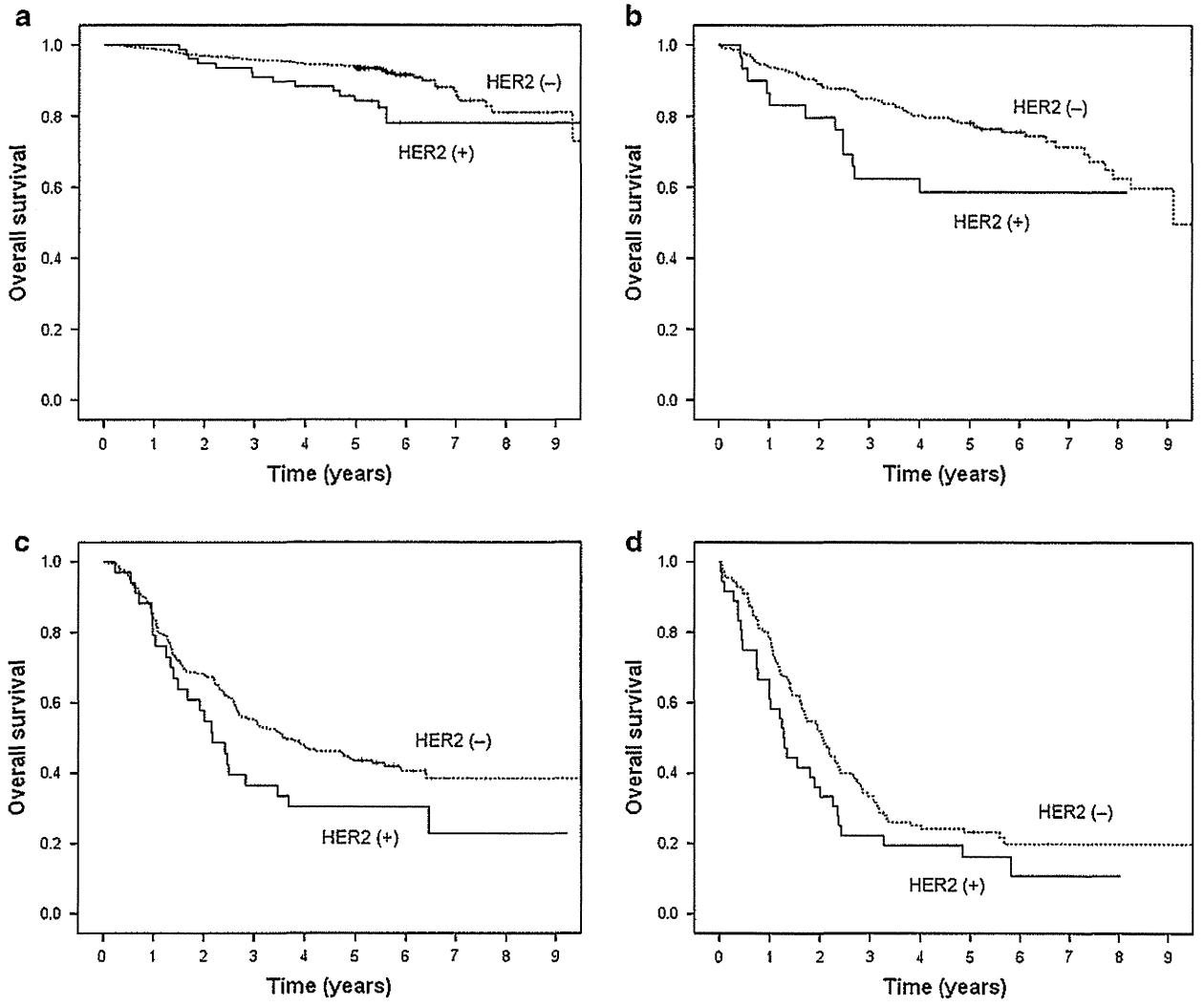

Fig. 2 Kaplan–Meier overall survival for patients with different stages of gastric cancer: **a** 482 patients with stage I cancer; **b** 255 patients with stage II cancer; **c** 263 patients with stage III cancer; **d** 148 patients with stage IV cancer

Table 2 Multivariate overall survival analysis

Variables	Category	HR (95 % CI)	*P*
Age (years)	≥70	2.05 (1.67–2.51)	<0.001
Sex	Male	1.23 (0.97–1.54)	0.085
Location	Upper	1.25 (1.00–1.56)	0.055
Histological type	Diffuse type	1.07 (0.86–1.33)	0.57
pT	T3-4	3.23 (2.44–4.27)	<0.001
pN	N1-3	2.90 (2.22–3.77)	<0.001
Adjuvant chemotherapy	Yes	1.24 (1.00–1.55)	0.055
HER2	Positive	1.96 (1.51–2.55)	<0.001

CI confidence interval, *HR* hazard ratio

HER2 positivity rate (13.6 %) in the study of Terashima et al. was relatively low compared with the rates obtained in other large-scale studies, although whether this explains

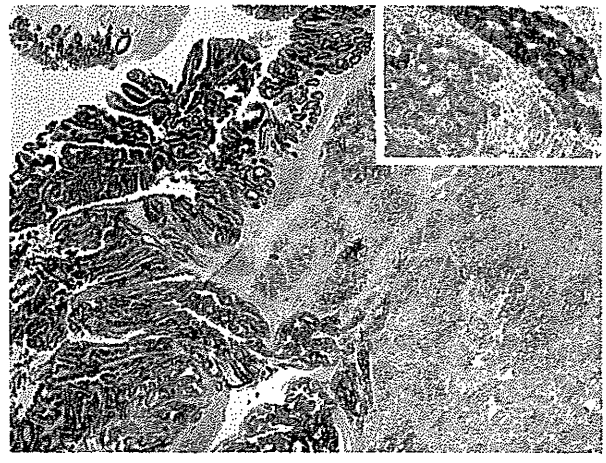

Fig. 3 Intratumoral heterogeneity of HER2 overexpression

Table 3 Characteristics of 175 HER2-positive patients stratified by intratumoral HER2 heterogeneity

Characteristics	Homogeneous ($n = 43$)	Heterogeneous ($n = 132$)	P
Age (years)			
Median	66	68	0.27
Range	46–80	37–87	
Sex			
Male	34	97	0.46
Female	9	35	
Location			
Upper	13	32	0.44
Middle/ lower	30	100	
Histological type			
Intestinal type	40	99	0.011
Diffuse type	3	33	
pT			
T1	13	60	0.008
T2	5	19	
T3	15	16	
T4	10	37	
pN			
N0	17	69	0.17
N1	6	16	
N2	11	16	
N3	9	31	
Pathological stage			
I	14	62	0.32
II	8	21	
III	9	26	
IV	12	23	
HER2 IHC score			
2+	0	18	0.011
3+	43	114	

IHC immunohistochemistry

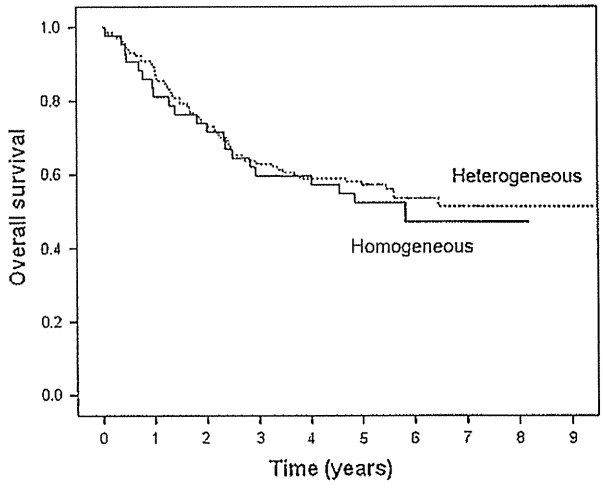

Fig. 4 Kaplan–Meier overall survival for 175 HER2-positive patients stratified by intratumoral HER2 heterogeneity

the difference in the results between the study of Terashima et al. and our study is unknown. The HER2 positivity rate in our study (15.7 %) was similar not only to that in the Western ToGA study (16.6 %), but also to that in a recent Japanese cohort study (15.5 %) [14, 15].

Some studies have evaluated the prognostic impact of HER2 expression also in unresectable or recurrent gastric cancer. Janjigian et al. [16] conducted a retrospective multicenter study examining HER2 expression in 381 Western patients with metastatic gastric or esophagogastric junction cancer, and reported that HER2 overexpression was not an independent prognostic factor. Shitara et al. [17] conducted a retrospective study in Japanese patients with unresectable or recurrent gastric cancer, and reported that the OS in 15 HER2-positive patients not receiving trastuzumab treatment was similar to that in 306 HER2-negative patients. Recently, a Chinese prospective cohort study showed that 51 HER2-positive patients receiving trastuzumab had prognoses comparable to those of 251 patients with HER2-negative advanced gastric cancer, whereas 47 HER2-positive patients not receiving trastuzumab had the poorest prognosis [18]. Although our large-scale study demonstrated that HER2 expression is an independent prognostic factor in resectable gastric cancer, the prognostic value of HER2 status in unresectable or recurrent gastric cancer is still unknown. Indeed, there were smaller differences between HER2-positive and HER2-negative patients in the stage IV cancer subgroup than in the stage I cancer, stage II cancer and stage III cancer subgroups. We anticipate that an ongoing cohort study evaluating HER2 expression in patients with unresectable or recurrent gastric cancer will clarify this issue.

Intratumoral HER2 heterogeneity is important because it may lead to inaccurate assessment of HER2 status and the consequent inappropriate choice of trastuzumab treatment. It has been reported that HER2 heterogeneity is more frequent in gastric cancer than in breast cancer [11]. Indeed, intratumoral HER2 heterogeneity by IHC was observed in three quarters of the HER2-positive tumors in our study. Lee et al.[19] reported that HER2 homogeneity conferred poorer survival than HER2 heterogeneity in a small-scale ($n = 64$) retrospective study. Our study also revealed that heterogeneity was more frequent in diffuse-type tumors and in tumors with and IHC score of 2+, and that there was no impact on prognosis. However, the impact on trastuzumab response remains unclear, so further studies to evaluate the therapeutic impact of HER2 heterogeneity are warranted.

To our knowledge, this is the first study to evaluate HER2 status in over 1,100 patients with gastric cancer. In conclusion, our multicenter large-scale study demonstrated that HER2 overexpression is an independent prognostic factor in gastric cancer patients. Although intratumoral HER2 heterogeneity was observed frequently, it did not affect prognosis at all.

Acknowledgments This work was supported by Chugai Pharmaceutical. The authors thank Y. Hayashi for her review of this report on behalf of Chugai Pharmaceutical.

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. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med. 2007;357:39–51.
3. Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. J Clin Oncol. 1993;11:1936–42.
4. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. J Clin Oncol. 2010;28:92–8.
5. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. 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, randomised controlled trial. Lancet. 2010;376:687–97.
6. Wang YL, Sheu BS, Yang HB, Lin PW, Chang YC. Overexpression of c-erb-B2 proteins in tumor and non-tumor parts of gastric adenocarcinoma—emphasis on its relation to H. pylori infection and clinicohistological characteristics. Hepatogastroenterology. 2002;49:1172–6.
7. Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL, Da Costa WL Jr, et al. Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcimomas: HER2 and HER3 are predictors of poor outcome. J Clin Oncol. 2011;29:3030–6.
8. Kim KC, Koh YW, Chang HM, Kim TH, Yook JH, Kim BS, et al. Evaluation of HER2 protein expression in gastric carcinomas: comparative analysis of 1,414 cases of whole-tissue sections and 595 cases of tissue microarrays. Ann Surg Oncol. 2011;18:2833–40.
9. Kataoka Y, Okabe H, Yoshizawa A, Minamiguchi S, Yoshimura K, Haga H, et al. HER2 expression and its clinicopathological features in resectable gastric cancer. Gastric Cancer. 2013;16:84–93.
10. Terashima M, Kitada K, Ochiai A, Ichikawa W, Kurahashi I, Sakuramoto S, et al. Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. Clin Cancer Res. 2012;18:5992–6000.
11. Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology. 2008;52:797–805.
12. Sobin LH, Gospodarowicz MK, Wittekind CH. TNM classification of malignant tumours. 7th ed. New York: Wiley-Blackwell; 2009.
13. Jørgensen JT, Hersom M. HER2 as a prognostic marker in gastric cancer—a systematic analysis of data from the literature. J Cancer. 2012;3:137–44.
14. Chung HC, Bang YJ. Human epidermal growth factor receptor 2 (HER2) in gastric cancer (GC): results of the ToGA trial screening programme and recommendations for HER2 testing. EJC Suppl. 2009;7(2):364.
15. Nishikawa K, Chin K, Nashimoto A. Result of HER2 status in Japanese metastatic gastric cancer–prospective cohort study (JFMC44-1101). J Clin Oncol 2012;30:(Suppl 4):10.
16. Janjigian YY, Werner D, Pauligk C, Steinmetz K, Kelsen DP, Jäger E, et al. Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: a European and USA International collaborative analysis. Ann Oncol. 2012;23:2656–62.
17. Shitara K, Yatabe Y, Matsuo K, Sugano M, Kondo C, Takahari D, et al. Prognosis of patients with advanced gastric cancer by HER2 status and trastuzumab treatment. Gastric Cancer. 2013;16:261–7.
18. Qiu MZ, Li Q, Wang ZQ, Liu TS, Liu Q, Wei XL, et al. HER2-positive patients receiving trastuzumab treatment have a comparable prognosis with HER2-negative advanced gastric cancer patients: a prospective cohort observation. Int J Cancer. 2014;134:2468–77.
19. Lee HE, Park KU, Yoo SB, Nam SK, Park DJ, Kim HH, et al. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. Eur J Cancer. 2013;49:1448–57.

Ann Surg Oncol (2014) 21:S584–S590
DOI 10.1245/s10434-014-3690-x

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

ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Prognostic Impact of Major Receptor Tyrosine Kinase Expression in Gastric Cancer

Yukinori Kurokawa, MD[1], Nariaki Matsuura, MD[2], Ryohei Kawabata, MD[3], Kazuhiro Nishikawa, MD[4], Chikara Ebisui, MD[5], Yuhki Yokoyama, PhD[2], Mohammed Nouri Shaker, MD[2], Takuya Hamakawa, MD[1], Tsuyoshi Takahashi, MD[1], Shuji Takiguchi, MD[1], Masaki Mori, MD, FACS[1], and Yuichiro Doki, MD[1]

[1]Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan; [2]Department of Molecular Pathology, Graduate School of Medicine, Osaka University, Osaka, Japan; [3]Department of Surgery, Sakai Municipal Hospital, Osaka, Japan; [4]Department of Surgery, Osaka General Medical Center, Osaka, Japan; [5]Department of Surgery, Suita Municipal Hospital, Osaka, Japan

ABSTRACT

Background. Various kinds of molecular targeted drugs to inhibit receptor tyrosine kinases (RTKs) have been recently developed. The relationship between the expression status of major RTKs and prognosis in gastric cancer remains unclear. We conducted a multicenter study to evaluate the prognostic impact of the expression of epidermal growth factor receptor (EGFR), c-Met, platelet-derived growth factor receptor (PDGFR), and c-Kit in gastric cancer.

Methods. This study included 153 gastric cancer patients who underwent gastrectomy at 9 institutions between 2000 and 2006. Expression status of EGFR, c-Met, PDGFR, and c-Kit were evaluated with immunohistochemistry (IHC) centrally. Overall survival based on RTK expression status was statistically compared. Cox multivariate analysis was conducted to adjust for potentially confounding factors.

Results. The positive rates for EGFR, c-Met, PDGFR, and c-Kit were 14.4, 24.8, 41.2, and 11.1 %, respectively. Significant interactions with expression status were observed for pathological N stage with EGFR; HER2-status with c-Met; tumor location, histology, and pathological N stage with PDGFR; and no examined variables with c-Kit. Concomitant HER2 positivity was observed for 0.7 % of tumors positive for EGFR, 3.9 % for c-Met, 4.6 % for PDGFR, and 1.3 % for c-Kit. There were some differences in overall survival between patients with or without RTK expression, but only c-Kit expression showed a significant survival difference in Cox multivariate analysis ($P = 0.046$).

Conclusions. Our multicenter study indicated that IHC expression of 4 RTKs had some prognostic impact and that c-Kit–positive status may be a significant indicator of good prognosis in gastric cancer patients.

Gastric cancer is the second leading cause of cancer death worldwide.[1] Complete resection of localized tumors is the mainstay of treatment, although recurrence and metastasis occur frequently. Given the progress in molecular technology, many researchers around the world are attempting to identify cancer driver genes. Receptor tyrosine kinases (RTKs) have been considered the most important targets; human epidermal growth factor receptor 2 (HER2) has already been identified as the key oncogenic driver of breast cancer.[2,3] Recent studies have shown that HER2 status is a significant prognostic and predictive factor for gastric cancer as well.[4–6]

However, in published studies the HER2-positive rate was only around 16 % in gastric cancer patients, so other molecular markers that predict prognosis would be the next targets.[7,8] Here, we investigated 4 oncogene-associated RTKs for which molecular targeted agents are available to treat various malignancies: epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (HGFR), stem cell factor receptor (SCFR), and platelet-derived growth factor receptor (PDGFR). EGFR, HGFR, and SCFR are proteins encoded by proto-oncogenes *c-erbB-1*, *c-met*, and *c-kit*, respectively. PDGFR is a receptor for PDGF, and PDGF is a product of the oncogene *sis*. Since these receptors are overexpressed in a variety of malignancies, molecular targeted drugs that inhibit these

First Received: 12 January 2014;
Published Online: 18 April 2014

Y. Kurokawa, MD
e-mail: ykurokawa@gesurg.med.osaka-u.ac.jp

RTKs have been developed and shown to have therapeutic impact. However, the impact of the expression of these RTKs on prognosis in gastric cancer remains unknown. Thus, we conducted immunohistochemical (IHC) analysis of these 4 molecules to clarify their prognostic significance in gastric cancer.

PATIENTS AND METHODS

Patient Population

This study included 153 gastric cancer patients who underwent gastrectomy between 2000 and 2006 at the 9 institutions comprising the Osaka University Clinical Research Group for Gastroenterological Surgery. Patients who underwent preoperative chemotherapy or radiation therapy were excluded. No patients underwent targeted therapy with any RTK inhibitors, even after recurrence. All tumors were histologically diagnosed as adenocarcinoma of the stomach. Pathological staging was based on the seventh edition of the International Union Against Cancer (UICC) TNM Classification.[9] This study was approved by the institutional review board of each participating institution.

IHC Analysis

All tumor tissues were fixed in 10–20 % formalin for 24–72 h in each institution. Archived formalin-fixed, paraffin-embedded specimens were shipped to the institution performing central pathological review. Each specimen was composed of several blocks of the tumor from the proximal and distal borders and adjacent normal gastric tissue. IHC studies were carried out on 4-μm-thick tissue sections. The primary antibodies included mouse monoclonal anti-EGFR (clone 3C6, Ventana Medical Systems, Tucson, AZ), rabbit monoclonal anti–total c-Met (clone SP44, Ventana Medical Systems), mouse monoclonal anti–c-Kit (clone 9.7, Ventana Medical Systems), and rabbit polyclonal anti-PDGFR-a (clone C20, Santa Cruz Biotechnology, Inc., Dallas, TX). Immunostaining was performed using a Ventana BenchMark XT autoimmunostainer with the I-VIEW DAB Universal Kit (Ventana Medical Systems). Antigen retrieval was performed by autoclaving in EDTA-base buffer, pH 8.5 at 98 °C for 60 min for c-Met and c-Kit, or by treating with protease I at 37 °C for 8 min for EGFR and PDGFR. Shortly after blocking with endogenous peroxidase, the sections were incubated with primary antibodies at 37 °C for 32 min, followed by incubating with a mixture of goat biotinylated anti-mouse IgG and IgM and anti-rabbit IgG antibodies for 8 min, and then peroxidase-conjugated streptavidin for 8 min. Visualization was performed after developing in 3,3′-diaminobenzidine (DAB) solution for 8 min, followed by signal amplification with copper sulfate for 4 min and counterstaining with hematoxylin for 4 min. Tissue samples consisting of skin keratinocytes, hepatocellular carcinoma, gastrointestinal stromal tumor, and dermis were used as positive controls for EGFR, c-Met, c-Kit, and PDGFR, respectively. Slides processed without the primary antibodies were used as negative controls. All instances of improper staining were repeated and properly examined.

Evaluation

Central pathologists independently performed IHC analysis without prior knowledge of the patient's clinical data. Evaluation of the expression status of EGFR, c-Met, PDGFR, and c-Kit was based on previous studies.[10–13] In brief, tumors with complete or basolateral membranous reactivity with strong intensity in $\geq$10 % of tumor cells by IHC were defined as EGFR-positive (Fig. 1a).[10] c-Met positivity was defined as having at least 50 % of tumor cells stained by IHC with strong or moderate intensity (membrane or cytoplasmic staining, or both) (Fig. 1b).[11] PDGFR reactivity was scored based on the extent of staining on cancer cell membranes or in the cytoplasm, and the threshold of positivity for PDGFR expression was 25 % staining (Fig. 1c).[12] For c-Kit expression, tumors strongly stained with or without membrane staining in 10 % or more of cancer cells were defined as c-Kit–positive (Fig. 1d).[13]

Statistical Analysis

The relationship between EGFR, c-Met, PDGFR, or c-Kit expression and clinicopathological factors was analyzed using the Chi square test for categorical variables and the Mann–Whitney *U* test for continuous variables. Overall survival (OS) was defined as the interval from the date of surgery to the date of death from any cause. The survival rate was estimated using the Kaplan–Meier method, and survival was compared using the log-rank test. The hazard ratio (HR) for death in each marker-positive patient was estimated using Cox proportional hazards models. Multivariate Cox regression analyses were performed to adjust for potential confounding factors. *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics software, version 20 (IBM Corp., Armonk, NY).

RESULTS

Expression Status

The relationship between the clinicopathological characteristics and the expression status of EGFR, c-Met,

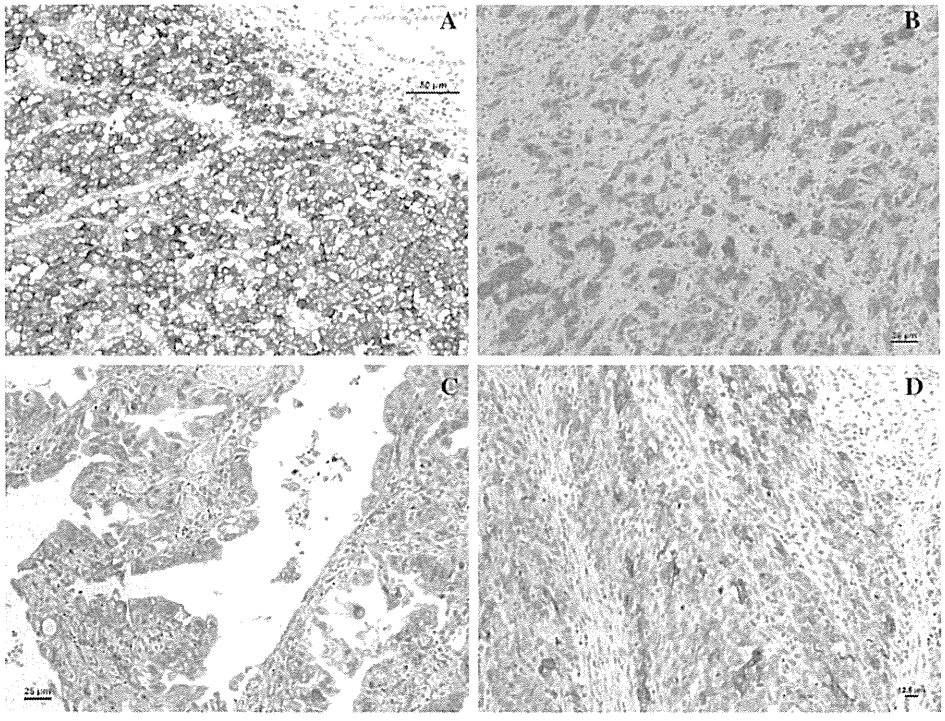

FIG. 1 Tumor specimens with strong expression of **a** EGFR, **b** c-Met, **c** PDGFR, and **d** c-Kit

PDGFR, and c-Kit was analyzed (Table 1). Based on IHC testing, the EGFR-positive rate for all 153 tumors was 14.4 % (22 of 153). The EGFR-positive group had a significantly higher proportion of advanced N stage tumors ($P = 0.049$). There was 1 tumor that was positive for EFGR and HER2 (0.7 %).

The c-Met–positive rate was 24.8 % (38 of 153). The HER2-positive rate was significantly higher in c-Met–positive tumors than in c-Met–negative tumors ($P = 0.036$). There were 3.9 % (6 of 153) of tumors that were positive for both c-Met and HER2.

The PDGFR-positive rate was 41.2 % (63 of 153). Tumors located in the upper body of the stomach and differentiated type tumors had a significantly higher rate of PDGFR positivity ($P = 0.015$ and $P = 0.003$, respectively). Pathological N stage was also significantly correlated with PDGFR status ($P = 0.013$). There were 7 tumors positive for both PDGFR and HER2 (4.6 %).

The c-Kit–positive rate was 11.1 % (17 of 153). There were no significant factors associated with c-Kit expression. There were 2 tumors that were positive for both c-Kit and HER2 (1.3 %).

Survival

The OS of gastric cancer patients that were positive or negative for RTK expression were compared (Fig. 2a–d). The 5-year OS rates in the positive and negative patients were: EGFR-positive, 16.4 %; EGFR-negative, 40.9 %; c-Met–positive, 23.0 %; c-Met–negative, 42.0 %; PDGFR-positive, 44.2 %; PDGFR-negative, 33.0 %; c-Kit–positive, 63.6 %; c-Kit–negative, 34.7 %. The HRs for death in patients with RTK-positive tumors were: 1.60 (95 % confidence interval [95 % CI] 0.94–2.71) (log-rank $P = 0.082$) for EGFR, 1.44 (95 % CI 0.91–2.27) (log-rank $P = 0.12$) for c-Met, 0.71 (95 % CI 0.46–1.10) (log-rank $P = 0.13$) for PDGFR, and 0.42 (95 % CI 0.17–1.02) (log-rank $P = 0.048$) for c-Kit.

Cox multivariate analysis that included the expression status for these 4 RTKs and 8 clinicopathological factors including HER2 status revealed that age, pathological T stage, pathological N stage, HER2 status, and c-Kit status were significant prognostic factors ($P = 0.046$) (Table 2).

DISCUSSION

Greater understanding of carcinogenesis and cancer progression on the molecular level has led to a new paradigm for anticancer therapies: targeting specific molecules associated with the growth of cancer cells. Since neoplastic cells frequently show "addiction" to activated oncogenes, such oncogene products comprise the most promising group of drug targets to date. Among these, RTKs are the most important targets because they promote cell proliferation, differentiation, migration, and inhibition of apoptosis via downstream signaling pathways. Activation

TABLE 1 Characteristics of 153 patients evaluating expressions of EGFR, c-Met, PDGFR, and c-Kit

Characteristics	EGFR			c-Met		
	Positive ($n = 22$)	Negative ($n = 131$)	P value	Positive ($n = 38$)	Negative ($n = 115$)	P value
Age (years)						
Median	69.5	68	0.53	68	68	0.61
Range	35–80	37–98		35–98	37–89	
Sex						
Male	18	86	0.13	26	78	0.95
Female	4	45		12	37	
Location						
Upper	6	45	0.52	9	42	0.15
Middle/lower	16	86		29	73	
Histology						
Differentiated	4	31	0.57	8	27	0.76
Undifferentiated	18	100		30	88	
pT						
T1	0	6	0.58	1	5	0.96
T2	1	13		4	10	
T3	5	31		9	27	
T4	16	81		24	73	
pN						
N0	1	36	0.049	6	31	0.40
N1	3	28		7	24	
N2	6	22		7	21	
N3	12	45		18	39	
HER2						
Positive	1	11	0.53	6	6	0.036
Negative	21	120		32	109	
Characteristics	PDGFR			c-Kit		
	Positive ($n = 63$)	Negative ($n = 90$)	P value	Positive ($n = 17$)	Negative ($n = 136$)	P value
Age (years)						
Median	69	67.5	0.56	69	68	0.25
Range	37–89	35–98		43–89	35–98	
Sex						
Male	42	62	0.77	12	92	0.81
Female	21	28		5	44	
Location						
Upper	28	23	0.015	6	45	0.86
Middle/lower	35	67		11	91	
Histology						
Differentiated	22	13	0.003	4	31	0.95
Undifferentiated	41	77		13	105	
pT						
T1	3	3	0.36	1	5	0.15
T2	8	6		4	10	
T3	17	19		4	32	
T4	35	62		8	89	

TABLE 1 continued

Characteristics	PDGFR			c-Kit		
	Positive ($n = 63$)	Negative ($n = 90$)	P value	Positive ($n = 17$)	Negative ($n = 136$)	P value
pN						
N0	19	18	0.013	5	32	0.29
N1	17	14		6	25	
N2	13	15		2	26	
N3	14	43		4	53	
HER2						
Positive	7	5	0.21	2	10	0.52
Negative	56	85		15	126	

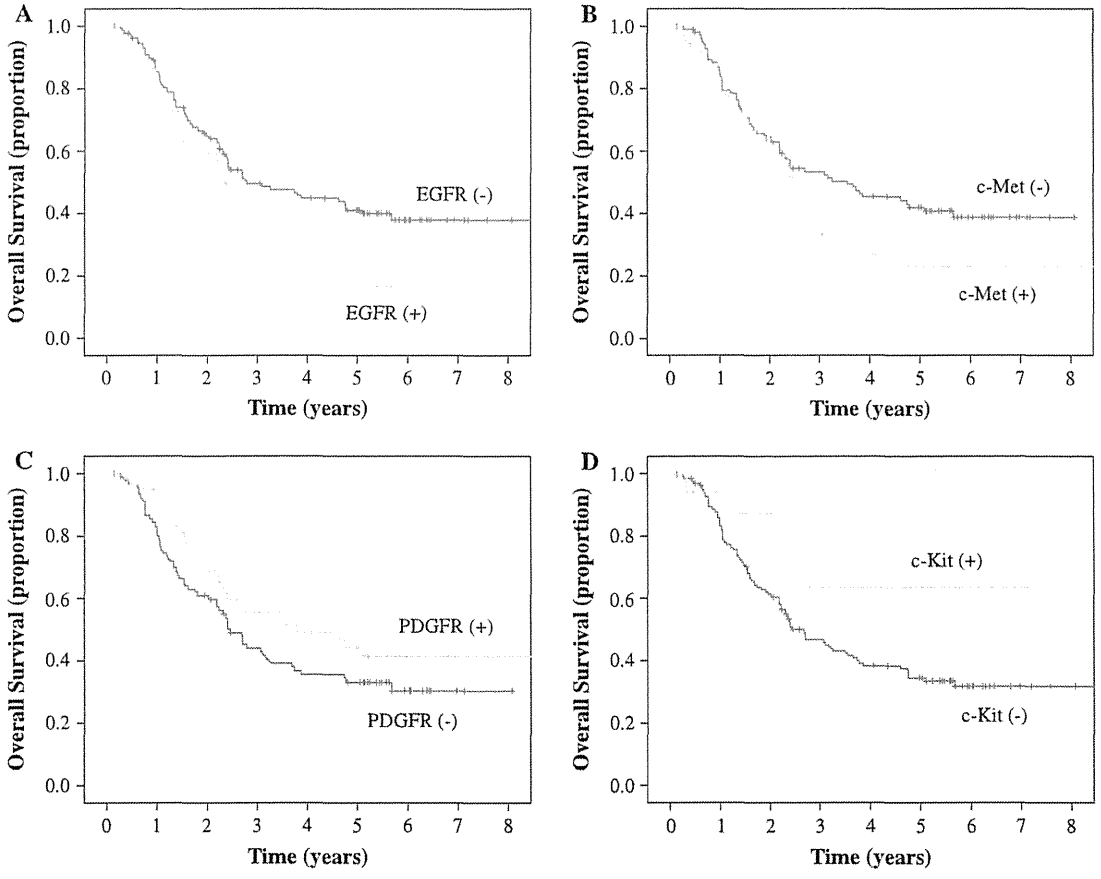

FIG. 2 Kaplan-Meier overall survival of gastric cancer patients who were positive or negative for **a** EGFR, **b** c-Met, **c** PDGFR, and **d** c-Kit

of these RTKs by mutation or overexpression is frequently detected in a variety of malignancies. Our multicenter study evaluating the prognostic impact of 4 important RTKs, i.e., EGFR, c-Met, PDGFR, and c-Kit, revealed differences in survival between patients with and without expression of each, and c-Kit expression has the largest impact on prognosis.

EGFR is an RTK expressed on the cell membrane that belongs to the ErbB family. Once activated, EGFR initiates a complex intracellular signal transduction cascade promoting cancer cell division, migration, angiogenesis, and inhibition of apoptosis.[14] EGFR has been found to play a major role in carcinogenesis, and its overexpression has generally been associated with disease.[15] On the other

TABLE 2 Multivariate overall survival analyses

Variable	Category	Hazard ratio (95 % CI)	*P* value
Age (years)	≥70	1.99 (1.28–3.08)	0.002
Sex	Female	1.22 (0.78–1.93)	0.38
Location	Upper	1.40 (0.87–2.25)	0.17
Histology	Undifferentiated	1.01 (0.55–1.86)	0.98
pT	T3–4	11.75 (1.57–88.16)	0.017
pN	N1–3	2.07 (1.04–4.10)	0.038
Adjuvant chemotherapy	Yes	1.49 (0.93–2.38)	0.096
HER2	Positive	2.99 (1.38–6.51)	0.006
EGFR	Positive	1.32 (0.75–2.32)	0.34
c-Met	Positive	1.37 (0.83–2.25)	0.22
PDGFR	Negative	1.46 (0.90–2.38)	0.13
c-Kit	Negative	2.65 (1.02–6.90)	0.046

hand, c-Met, a hepatocyte growth factor receptor, belongs to a family of tyrosine kinase growth factor receptors. c-Met signaling promotes tumor cell growth and motility. In gastric cancer, some previous studies reported that overexpression of EGFR or c-Met was a prognostic indicator.[16–22] In our study, overexpression of EGFR or c-Met tended to be associated with poor prognosis, but there were no significant effects after adjustment for potentially confounding factors with multivariate analysis. Most previous studies may have arrived at different results for EGFR and c-Met because they did not involve multivariate analysis.

PDGFR is a transmembrane RTK activated by PDGF. Both are implicated in a variety of physiologic and pathologic processes, including cell growth.[23,24] Some in vitro and in vivo studies have reported that expression of PDGFR in gastric cancer stromal cells is correlated with tumor progression.[25,26] The c-Kit proto-oncogene encodes a type III RTK; 1 of its ligands is stem cell factor. The activated interaction between c-Kit and stem cell factor is essential for the development of melanocytes, erythrocytes, germ cells, mast cells, and interstitial cells of Cajal.[27] Sung et al. [28] reported that c-Kit–positive cells were located mainly in the stroma around the repair zone of the glands in chronic gastritis and may be associated with carcinogenesis in human gastric mucosa. PDGFR and c-Kit expression are considered important prognostic factors in gastrointestinal stromal tumors and other malignancies, but there was no study that has reported a close relationship between expression status and prognosis in gastric cancer patients.[29–36] Our study evaluated the prognostic impact of PDGFR and c-Kit in gastric cancer and found that c-Kit status was a significant prognostic factor even with multivariate analysis.

In conclusion, our multicenter study indicated that expression of 4 RTKs as assessed by IHC has some prognostic impact and that c-Kit-positive status was a significant indicator of good prognosis in gastric cancer patients. To the best of our knowledge, this is the first study to reveal the prognostic impact of c-Kit expression in gastric cancer. Although this study was limited by its small sample size and retrospective nature, we expect that our study will contribute to the establishment of new molecular targeted therapies in the field of gastric cancer.

ACKNOWLEDGMENT This study was supported by Chugai Pharmaceutical.

CONFLICT OF INTEREST All authors have no conflicts of interest or financial ties to disclose.

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. Seshadri R, Firgaira FA, Horsfall DJ, McCaul K, Setlur V, Kitchen P. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian Breast Cancer Study Group. *J Clin Oncol*. 1993;11:1936–42.
3. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. *J Clin Oncol*. 2010;28:92–8.
4. Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL, da Costa WL Jr, et al. Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 are predictors of poor outcome. *J Clin Oncol*. 2011;29,3030–6.
5. Kim KC, Koh YW, Chang HM, Kim TH, Yook JH, Kim BS, et al. Evaluation of HER2 protein expression in gastric carcinomas: comparative analysis of 1,414 cases of whole-tissue sections and 595 cases of tissue microarrays. *Ann Surg Oncol*. 2011; 18:2833–40.
6. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. 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, randomised controlled trial. *Lancet*. 2010;376:687–97.
7. Chung HC, Bang YJ, Xu JM, Lordick F, Sawaki A, Lipatov O, et al. Human epidermal growth factor receptor 2 (HER2) in gastric cancer (GC): results of the ToGA trial screening programme and recommendations for HER2 testing. No. 6511. EJC Supplements. 2009;7:364. ECCO 15/ESMO 34.
8. Nishikawa K, Chin K, Nashimoto A, Miki A, Miwa H, Tsuburaya A, et al. Result of HER2 status in Japanese metastatic gastric cancer: Prospective cohort study (JFMC44-1101). *J Clin Oncol*. 2012;30: suppl 34; abstr 10.
9. Sobin LH, Gospodarowicz MK, Wittekind CH. TNM classification of Malignant Tumours. 7th ed. New York: Wiley-Blackwell, 2009.
10. Atmaca A, Werner D, Pauligk C, Steinmetz K, Wirtz R, Altmannsberger HM, et al. The prognostic impact of epidermal

growth factor receptor in patients with metastatic gastric cancer. *BMC Cancer*. 2012;12:524.
11. Spigel DR, Edelman MJ, Mok T, O'Byrne K, Paz-Ares L, Yu W, et al. Treatment rationale study design for the MetLung trial: a randomized, double-blind phase III study of onartuzumab (MetMAb) in combination with erlotinib versus erlotinib alone in patients who have received standard chemotherapy for stage IIIB or IV met-positive non-small-cell lung cancer. *Clin Lung Cancer*. 2012;13:500–4.
12. Varela M, Ranuncolo SM, Morand A, Lastiri J, De Kier Joffé EB, Puricelli LI, et al. (2004) EGF-R and PDGF-R, but not bcl-2, overexpression predict overall survival in patients with low-grade astrocytomas. *J Surg Oncol*. 86:34–40.
13. Massard C, Voigt JJ, Laplanche A, Culine S, Lortholary A, Bugat R, et al. Carcinoma of an unknown primary: are EGF receptor, Her-2/neu, and c-Kit tyrosine kinases potential targets for therapy? *Br J Cancer*. 2007;97:857–61.
14. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res*. 2001;7:2958–70.
15. Lemoine NR, Jain S, Silvestre F, Lopes C, Hughes CM, McLelland E, et al. Amplification and overexpression of the EGF receptor and c-erbB-2 proto-oncogenes in human stomach cancer. *Br J Cancer*. 1991;64:79–83.
16. Garcia I, Vizoso F, Martin A, Sanz L, Abdel-Lah O, Raigoso P, et al. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. *Ann Surg Oncol*. 2003;10:234–41.
17. Hayashi M, Inokuchi M, Takagi Y, Yamada H, Kojima K, Kumagai J, et al. High expression of HER3 is associated with a decreased survival in gastric cancer. *Clin Cancer Res*. 2008;14: 7843–9.
18. Kim MA, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WH. EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. *Histopathology*. 2008;52: 738–46.
19. Terashima M, Kitada K, Ochiai A, Ichikawa W, Kurahashi I, Sakuramoto S, et al. Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. *Clin Cancer Res*. 2012;18:5992–6000.
20. Nakajima M, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, et al. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer*. 1999;85:1894–902.
21. Huang TJ, Wang JY, Lin SR, Lian ST, Hsieh JS. Overexpression of the c-met protooncogene in human gastric carcinoma—correlation to clinical features. *Acta Oncol*. 2001;40:638–43.
22. Drebber U, Baldus SE, Nolden B, Grass G, Bollschweiler E, Dienes HP, et al. The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. *Oncol Rep*. 2008;19:1477–83.
23. Hart CE, Forstrom JW, Kelly JD, Seifert RA, Smith RA, Ross R, et al. Two classes of PDGF receptor recognize different isoforms of PDGF. *Science*. 1988;240:1529–31.
24. Yao R, Cooper GM. Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science*. 1995;267:2003–6.
25. Sumida T, Kitadai Y, Shinagawa K, Tanaka M, Kodama M, Ohnishi M, et al. Anti-stromal therapy with imatinib inhibits growth and metastasis of gastric carcinoma in an orthotopic nude mouse model. *Int J Cancer*. 2011;128:2050–62.
26. Kodama M, Kitadai Y, Sumida T, Ohnishi M, Ohara E, Tanaka M, et al. Expression of platelet-derived growth factor (PDGF)-B and PDGF-receptor β is associated with lymphatic metastasis in human gastric carcinoma. *Cancer Sci*. 2010;101:1984–9.
27. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science*. 1998;279:577–80.
28. Sung R, Kim YC, Yun HY, Choi W, Kim HS, Kim H, et al. Interstitial cells of Cajal (ICC)-like-c-Kit positive cells are involved in gastritis and carcinogenesis in human stomach. *Oncol Rep*. 2011;26:33–42.
29. Henriksen R, Funa K, Wilander E, Bäckström T, Ridderheim M, Oberg K. Expression and prognostic significance of platelet-derived growth factor and its receptors in epithelial ovarian neoplasms. *Cancer Res*. 1993;53:4550–4.
30. Paulsson J, Sjöblom T, Micke P, Pontén F, Landberg G, Heldin CH, et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. *Am J Pathol*. 2009;175:334–41.
31. Yuzawa S, Kano MR, Einama T, Nishihara H. PDGFRβ expression in tumor stroma of pancreatic adenocarcinoma as a reliable prognostic marker. *Med Oncol*. 2012;29:2824–30.
32. Chu JS, Ge FJ, Zhang B, Wang Y, Silvestris N, Liu LJ, et al. Expression and prognostic value of VEGFR-2, PDGFR-β, and c-Met in advanced hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2013;32:16.
33. Chung CY, Yeh KT, Hsu NC, Chang JH, Lin JT, Horng HC, et al. Expression of c-kit protooncogene in human hepatocellular carcinoma. *Cancer Lett*. 2005;217:231–6.
34. Tsutsui S, Yasuda K, Suzuki K, Takeuchi H, Nishizaki T, Higashi H, et al. A loss of c-kit expression is associated with an advanced stage and poor prognosis in breast cancer. *Br J Cancer*. 2006; 94:1874–8.
35. Camps C, Sirera R, Bremnes RM, Garde J, Safont MJ, Blasco A, et al. Analysis of c-kit expression in small cell lung cancer: prevalence and prognostic implications. *Lung Cancer*. 2006; 52:343–7.
36. de Melo Maia B, Lavorato-Rocha AM, Rodrigues IS, Baiocchi G, Cestari FM, Stiepcich MM, et al. (2012) Prognostic significance of c-KIT in vulvar cancer: bringing this molecular marker from bench to bedside. *J Transl Med*. 10:150.

Gastric Cancer (2014) 17:514–521
DOI 10.1007/s10120-013-0294-2

ORIGINAL ARTICLE

Validity of response assessment criteria in neoadjuvant chemotherapy for gastric cancer (JCOG0507-A)

Yukinori Kurokawa · Taro Shibata · Mitsuru Sasako · Takeshi Sano · Akira Tsuburaya · Yoshiaki Iwasaki · Haruhiko Fukuda

Received: 14 May 2013 / Accepted: 8 August 2013 / Published online: 3 September 2013

Abstract
Background Neoadjuvant chemotherapy may improve outcomes in gastric cancer. Tumor responses can be evaluated with RECIST, Japanese Classification of Gastric Carcinoma (JCGC), and histological criteria. These approaches have not yet been compared.
Methods We analyzed two phase II trials of neoadjuvant chemotherapy using S-1 plus cisplatin. JCOG0210 included patients with linitis plastica and large ulcero-invasive tumors, whereas JCOG0405 comprised those with paraaortic or bulky lymph node metastases. Radiologic evaluations were conducted using RECIST in JCOG0405 and JCGC criteria in JCOG0210, because the latter included many patients without measurable lesions. A histological responder was defined as a patient in whom one third or more of the tumor was affected. The hazard ratios (HR) for death between responders and non-responders and response rate differences between short- and long-term survivors were estimated.
Results In JCOG0210 ($n = 49$), HR was 0.54 in JCGC responders ($P = 0.059$) and 0.40 in histological responders ($P = 0.005$). The difference in response rates between short- and long-term survivors using histological criteria (34 %, $P = 0.023$) was greater than that using JCGC criteria (24 %, $P = 0.15$). In JCOG0405 ($n = 51$), HR was 0.67 in RECIST responders ($P = 0.35$) and 0.39 in histological responders ($P = 0.030$). In short- and long-term survivors, respectively, RECIST response rates were 62 and 67 % ($P = 0.77$), whereas histological response rates were 33 and 63 % ($P = 0.048$).
Conclusions Histological criteria showed higher response assessment validity than RECIST or JCGC criteria and yielded the best surrogate endpoint for overall survival.

Keywords RECIST · JCGC · JCOG

Y. Kurokawa (✉)
Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2-E2 Yamadaoka, Suita Osaka, Japan
e-mail: ykurokawa@gesurg.med.osaka-u.ac.jp

T. Shibata · H. Fukuda
Japan Clinical Oncology Group Data Center, National Cancer Center, Tokyo, Japan

M. Sasako
Department of Upper Gastrointestinal Surgery, Hyogo College of Medicine, Hyogo, Japan

T. Sano
Department of Surgery, Cancer Institute Hospital, Tokyo, Japan

A. Tsuburaya
Department of Surgery, Kanagawa Cancer Center, Kanagawa, Japan

Y. Iwasaki
Department of Surgery, Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, Tokyo, Japan

Introduction

Gastric cancer is the second-leading cause of cancer deaths worldwide and the most common cancer in Japan and Korea [1]. Although surgery is the standard treatment for resectable gastric cancer [2, 3], the prognosis of patients with advanced tumors is poor [4]. In particular, linitis plastica (Borrmann type 4) and large ulcero-invasive-type (Borrmann type 3) tumors, as well as those with paraaortic nodal metastases or bulky lymph node metastases, have extremely poor outcomes even after curative resection [5, 6]. For these advanced tumors, neoadjuvant chemotherapy