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ORIGINAL ARTICLE

Prognostic impact of HER2, EGFR, and c-MET status on overall survival of advanced gastric cancer patients

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Abstract

Background This study was conducted to investigate whether human epidermal growth factor receptor 2 (HER2) status, epidermal growth factor receptor (EGFR) status, and c-MET status are independent prognostic factors for advanced gastric cancer patients who received standard chemotherapy.

Method Unresectable or recurrent gastric or gastroesophageal junction cancer patients with histologically confirmed adenocarcinoma treated with S-1 plus cisplatin as first-line chemotherapy were eligible. Formalin-fixed paraffin-embedded tumor samples were examined for HER2, EGFR, and c-MET status using immunohistochemistry (IHC). Additionally, gene amplification was examined using fluorescent in situ hybridization (FISH) for HER2. Positivity was defined as an IHC score of 3+ or an IHC score of 2+/FISH positive for HER2, and an IHC score of 2+ or 3+ for both EGFR and c-MET.

Results Of the 293 patients from nine institutions, 43 (15 %) were HER2 positive, 79 (27 %) were EGFR positive, and 120 (41 %) were c-MET positive. Ten patients (3 %) showed positive co-expression of HER2, EGFR, and c-MET. After a median follow-up time of 58.4 months with 280 deaths, there was no significant difference in overall survival (OS) in terms of HER2 and EGFR status. However, there was a significant difference in OS between c-MET-positive and c-MET-negative patients [median, 11.9 months vs 14.2 months; hazard ratio, 1.31 (95 % confidence interval, 1.03–1.67); log-rank $P = 0.024$].

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Multivariate analysis also showed that c-MET positivity was still a prognostic factor for OS [hazard ratio, 1.30 (95 % confidence interval, 1.02–1.67); $P = 0.037$].

Conclusions The study suggested that c-MET-positive status had poor prognostic value. These data could be used as the basis for future clinical trials for targeting agents for advanced gastric cancer patients.

Keywords c-MET · Epidermal growth factor receptor · Gastric cancer · Gastroesophageal junction cancer · Human epidermal growth factor receptor 2

Introduction

Human epidermal growth factor receptor 2 (HER2) overexpression has been observed in 9–38 % of gastric cancer patients, and occurs more frequently in gastroesophageal junction (GEJ) and intestinal-type tumors [1]. Treatment with the anti-HER2 monoclonal antibody trastuzumab has been proven to achieve improved survival in HER2-positive advanced gastric cancer (AGC) patients; other new agents such as trastuzumab emtansine and pertuzumab are under investigation [2]. Besides HER2, new agents targeting epidermal growth factor receptor (EGFR) and c-MET have been extensively investigated in gastric cancer [3–5].

HER2 status as a prognostic factor for gastric cancer has been intensively investigated [1]. Although some studies have reported that HER2-positive status was a poor prognostic factor [6, 7], others have reported that it was a favorable prognostic factor or was not a significant prognostic factor [8–10]. However, HER2 diagnosis in these studies was based on only immunohistochemistry (IHC) without the standardized scoring system for gastric cancer [2]; most studies were conducted in resected gastric cancer patients and included a wide range of stages, from early gastric cancer to AGC. Therefore, the prognostic impact of HER2 status on overall survival (OS) in AGC

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patients treated with standard chemotherapy without trastuzumab for first-line treatment remains controversial.

The prognostic impact and clinicopathological features of EGFR and c-MET have also been studied. EGFR overexpression, which was observed in 27–44 % of gastric cancer patients, has been generally reported to be a poor prognostic factor [11–14]. The correlation between EGFR status and clinicopathological characteristics has not been elucidated. It has been reported that EGFR-positive status was frequently associated with the following factors: noncuratively treated gastric cancer [11]; older age, moderately to poorly differentiated histological appearance, and higher-stage disease [12]; and recurrence after curative surgery and higher disease stages [13]. On the whole, c-MET overexpression, which was observed in 22–82 % of gastric cancer patients, has been reported to be associated with poor prognosis [15–21]; however, findings from a few other studies were contradictory [22, 23]. The correlation between c-MET overexpression and clinicopathological features has been reported in patients with disease with differentiated histological appearance [20, 24, 25], lymph node metastasis [15, 16, 23, 26], peritoneal metastasis [15–17], liver metastasis [27], and advanced clinical stage [18, 23, 24]. However, these studies investigated patients who underwent resection, and diagnosis of EGFR and c-MET status was not standardized. Therefore, the prognostic impact of EGFR and c-MET status regarding OS in AGC patients remains unclear; this is also the case for HER2.

Most of the previous studies have involved patients whose disease stages differed, and even studies that have investigated AGC have involved patients who received chemotherapy regimens that were not always standard therapy. To investigate prognosis and elucidate the clinicopathological characteristics, it was considered desirable that the target population should be AGC patients who had received standard therapy [28]. Therefore, we enrolled patients who had received S-1 plus cisplatin as first-line therapy. Our study was conducted to investigate whether HER2 status, EGFR status, and c-MET status are independent prognostic factors in AGC patients, and to elucidate the correlation between this expression status and clinicopathological characteristics.

Patients and methods

Patients

We retrospectively collected the clinical data and tumor tissue. The eligibility criteria were as follows: (1) histologically confirmed gastric or GEJ (type I–III tumor using the Siewert classification [29]) adenocarcinoma; (2) unresectable or recurrent cancer; (3) treated with S-1 plus

cisplatin without trastuzumab as first-line chemotherapy between January 2006 and March 2010 [30]; (4) age 20 years or older; (5) Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; and (6) available archived tumor sample. Exclusion criteria included patients' refusal of permission for the use of clinical data and tumor tissue samples, and the presence of other active malignancy.

Clinicopathology data

We retrospectively collected the following clinicopathology data: age, ECOG PS, sex, primary tumor site, disease status, history of gastrectomy and adjuvant chemotherapy, histological appearance, presence of measurable disease categorized using Response Evaluation Criteria in Solid Tumors version 1.0, location of metastatic sites, serum alkaline phosphatase (ALP) value at the baseline of first-line chemotherapy, OS and progression-free survival (PFS) from the initiation of the first-line chemotherapy, and details of second-line and later-line chemotherapy if available.

HER2, EGFR, and c-MET assay

Formalin-fixed paraffin-embedded tumor samples from eligible patients were examined for HER2 using IHC and fluorescent in situ hybridization (FISH), and for EGFR and c-MET using IHC.

HER2 IHC analysis was performed at SRL (Tokyo, Japan) using PATHWAY anti-HER2/neu (4B5) rabbit monoclonal primary antibody (Ventana Medical Systems, Tucson, AZ, USA). The intensity of the membrane staining was evaluated according to the HER2 scoring system for gastric cancer as reported previously [2]. Surgical specimen staining patterns were scored as follows: score 0, no reactivity or membranous reactivity in less than 10 % of tumor cells; score 1+, faint/barely perceptible membranous reactivity in 10 % or more of tumor cells or reactive only in part of their membrane; score 2+, weak to moderate complete or basolateral membranous reactivity in 10 % or more of tumor cells; and score 3+, moderate to strong complete or basolateral membranous reactivity in 10 % or more of tumor cells (Fig. 1a–d). They were scored for biopsy specimen staining patterns if the staining reactivity of each score was identified in a cluster of five or more tumor cells, irrespective of the percentage of tumor cells stained. A pathologist from SRL primarily determined the HER2 IHC score, and T. Kuwata confirmed the results. If there was discrepancy in the HER2 IHC score among pathologists, the final judgment was made after sufficient discussion between T. Kuwata and A. Ochiai. HER2 FISH analysis was performed at SRL using the PathVysion

HER2 DNA probe kit (Vysis, Downers Grove, IL, USA), according to the manufacturer's instructions. When the ratio of HER2 signal to chromosome 17 centromere signal was 2.0 or greater, the gene was considered as amplified (i.e., FISH positive). HER2 positivity was defined as IHC score 2+ with a positive FISH result or IHC score 3+ with any FISH result.

Immunohistochemical staining of EGFR and c-MET was performed automatically by means of Ventana BenchMark® ULTRA using CONFIRM anti-EGFR (3C6) primary antibody (Ventana Medical Systems) and CONFIRM anti-total c-MET (SP44) rabbit monoclonal primary antibody (Ventana Medical Systems) at the National Cancer Center Hospital East. The intensity of the membrane staining regarding EGFR was scored as previously reported [25]: score 0, no reactivity or membranous reactivity in less than 10 % of tumor cells; score 1+, faint/barely perceptible membranous reactivity in 10 % or more of tumor cells or cells reactive only in part of their membrane; score 2+, weak to moderate complete or basolateral membranous reactivity in 10 % or more of tumor cells; and score 3+, moderate to strong complete or basolateral membranous reactivity in 10 % or more of tumor cells (Fig. 1e–h). EGFR positivity was defined as an IHC score of 2+ or 3+. The intensity of the membrane staining regarding c-MET was evaluated as previously reported [31]. Staining patterns were scored as follows: score 0, no reactivity or less than 50 % of tumor cells with any membranous reactivity; score 1+, 50 % or more of tumor cells with weak or higher membranous reactivity but less than 50 % with moderate or higher membranous reactivity; score 2+, 50 % or more of tumor cells with moderate or higher membranous reactivity but less than 50 % with strong membranous reactivity; and score 3+, 50 % or more of tumor cells with strong membranous reactivity (Fig. 1i–l). We defined c-MET positivity as an IHC membrane staining intensity score of 2+ or 3+. Y. Kuboki and T. Kuwata determined the EGFR and c-MET IHC score.

Statistical analysis

The target accrual of this retrospective study was 300 patients, assuming that more than 40 HER2-positive patients would be enrolled when the proportion of HER2-positive patients was approximately 15 %. Differences in patient characteristics were assessed using Fisher's exact test. Survival curves were estimated using the Kaplan–Meier method; OS was calculated from the start of S-1 plus cisplatin administration to death or the last follow-up, and PFS was calculated from the start of S-1 plus cisplatin administration to disease progression assessed by each investigator, death, or the last tumor assessment. The log-rank test was used to evaluate the difference between two
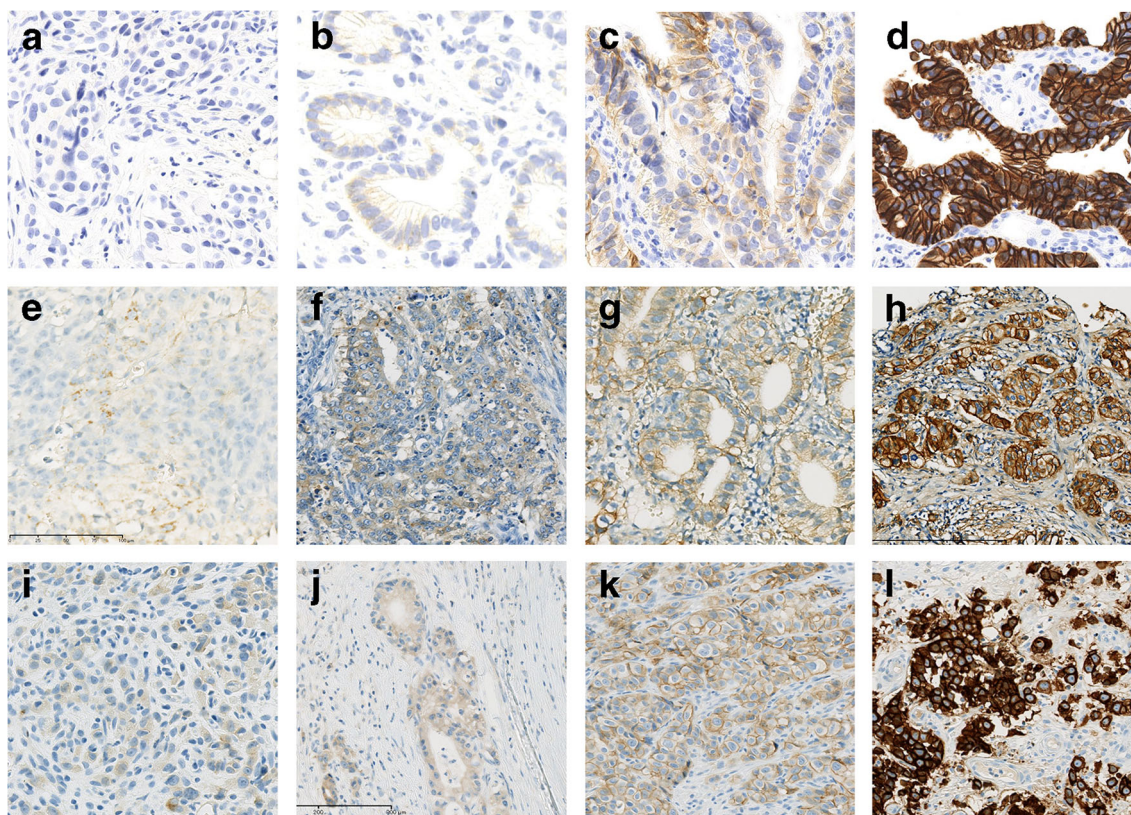
Fig. 1 Representative immunohistochemical staining of human epidermal growth factor receptor 2 (**a–d**), epidermal growth factor receptor (**e–h**), and c-MET (**i–l**): no membrane staining [immunohistochemistry (IHC) score 0; **a**, **e**, **i**], faint/barely visible staining intensity (IHC score 1+; **b**, **f**, **j**), weak to moderate staining intensity (IHC score 2+; **c**, **g**, **k**), and moderate to strong staining intensity (IHC score 3+; **d**, **h**, **l**)

survival curves. Multivariate Cox proportional hazard analysis was performed by the backward elimination with a stay level of 0.10. All P values were reported as two-tailed. Statistical analyses were performed using IBM® SPSS® Statistics version 21 (IBM, Armonk, NY, USA).

Ethical considerations

This study complied with Japanese ethical guidelines for epidemiological research and was approved by the Institutional Review Board and the director of each participating institution.

Results

Patients and follow-up

We enrolled 293 patients in this study from nine institutions within the planned period. Patient characteristics are detailed in Table 1. The baseline ALP value was not available for nine patients. Of the 293 patients, 213 (73 %) received second-line chemotherapy and 127 (43 %)

received third-line chemotherapy. After a median follow-up time of 58.4 months, 280 deaths, and 282 cases of progression or death were observed.

Human epidermal growth factor receptor 2

Of the 293 patients, 26 (9 %) had an IHC score of 3+, 17 (6 %) had an IHC score of 2+ and were FISH positive, and 43 (15 %) were HER2 positive (Table 2). The baseline patient characteristics were significantly different between HER2-positive and HER2-negative patients in terms of histological appearance ($P = 0.001$), presence of measurable disease ($P = 0.003$), number of metastatic sites ($P = 0.003$), presence of liver metastasis ($P = 0.003$), and ALP value ($P < 0.001$; Table S1). The location of the primary tumor site did not differ significantly between HER2-positive and HER2-negative patients ($P = 0.591$; Table S1). There was no significant difference in PFS {-median, 6.3 months [95 % confidence interval (CI), 5.2–7.3 months] vs 6.4 months [95 % CI, 5.7–7.1 months]; hazard ratio (HR), 1.08 [95 % CI, 0.77–1.50]; log-rank $P = 0.662$; Fig. 2a} and OS [median, 11.7 months (95 % CI, 7.4–16.0 months) vs 13.7 months (95 % CI,

Table 1 Baseline patient characteristics ($n = 293$)

Characteristics	Number	Percentage
Age (years)[a]		
<65	171	58
≥65	122	42
ECOG PS		
0	203	69
1–2	90	31
Sex		
Male	201	69
Female	92	31
Primary tumor site		
Stomach	263	90
Gastroesophageal junction	30	10
Disease status		
Unresectable	240	82
Recurrent	53	18
Previous gastrectomy		
Yes	100	34
No	193	66
Previous adjuvant chemotherapy		
Yes	18	6
No	275	94
Histology		
Differentiated	125	43
Undifferentiated	168	57
Measurable disease		
Yes	212	72
No	81	28
Number of metastatic sites		
0–1	146	50
≥2	147	50
Metastatic sites		
Liver	101	34
Peritoneal	145	49
Alkaline phosphatase level		
Normal	199	68
High	85	29
Not available	9	3

ECOG PS Eastern Cooperative Oncology Group performance status

[a] Median, 62 years, range, 28–82 years

Table 2 Human epidermal growth factor receptor 2 (*HER2*), epidermal growth factor receptor (*EGFR*), and c-MET data

|	Number	Percentage
HER2 IHC score		
0	224	76
1+	13	4
2+	30	10
3+	26	9
HER2 FISH		
HER2/CEN17 ≥ 2.0	54	18
HER2/CEN17 < 2.0	230	78
No result	9	3
EGFR IHC score		
0	155	53
1+	59	20
2+	40	14
3+	39	13
c-MET IHC score		
0	148	51
1+	25	9
2+	95	32
3+	25	9

CEN17 chromosome 17 centromere, *FISH* fluorescent in situ hybridization, *IHC* immunohistochemistry

Epidermal growth factor receptor

Of the 293 patients, 79 (27 %) were EGFR positive: 39 (13 %) had an IHC score of 3+, and 40 (14 %) had an IHC score of 2+ (Table 2). The baseline patient characteristics were significantly different between EGFR-positive and EGFR-negative patients in terms of disease status ($P = 0.016$; Table S1). There was no significant difference in PFS [median, 5.8 months (95 % CI, 4.3–7.4 months) vs 6.3 months (95 % CI, 5.8–6.9 months); HR, 1.03 (95 % CI, 0.79–1.34); log-rank $P = 0.825$; Fig. 2b] and OS [median, 11.9 months (95 % CI, 10.9–13.0 months) vs 14.2 months (95 % CI, 13.0–15.4 months); HR, 1.12 (95 % CI, 0.86–1.46); log-rank $P = 0.401$; Fig. 2e] between EGFR-positive and EGFR-negative patients. The proportions of patients who received second-line and third-line chemotherapy were not significantly different between EGFR-positive and EGFR-negative patients [71 % vs 73 % for second-line chemotherapy ($P = 0.661$) and 48 % vs 42 % for third-line chemotherapy ($P = 0.353$)].

c-MET

Of the 293 patients, 25 (9 %) had an IHC score of 3+, 95 (32 %) had an IHC score of 2+, and 120 (41 %) were c-MET-positive (Table 2). The baseline patient

12.4–14.9 months); HR, 1.09 (95 % CI, 0.78–1.51); log-rank $P = 0.630$; Fig. 2d] between HER2-positive and HER2-negative patients. The proportions of patients who received second-line and third-line chemotherapy were not significantly different between HER2-positive and HER2-negative patients [67 % v. 74 % for second-line chemotherapy ($P = 0.459$) and 35 % vs 45 % for third-line chemotherapy ($P = 0.247$)].
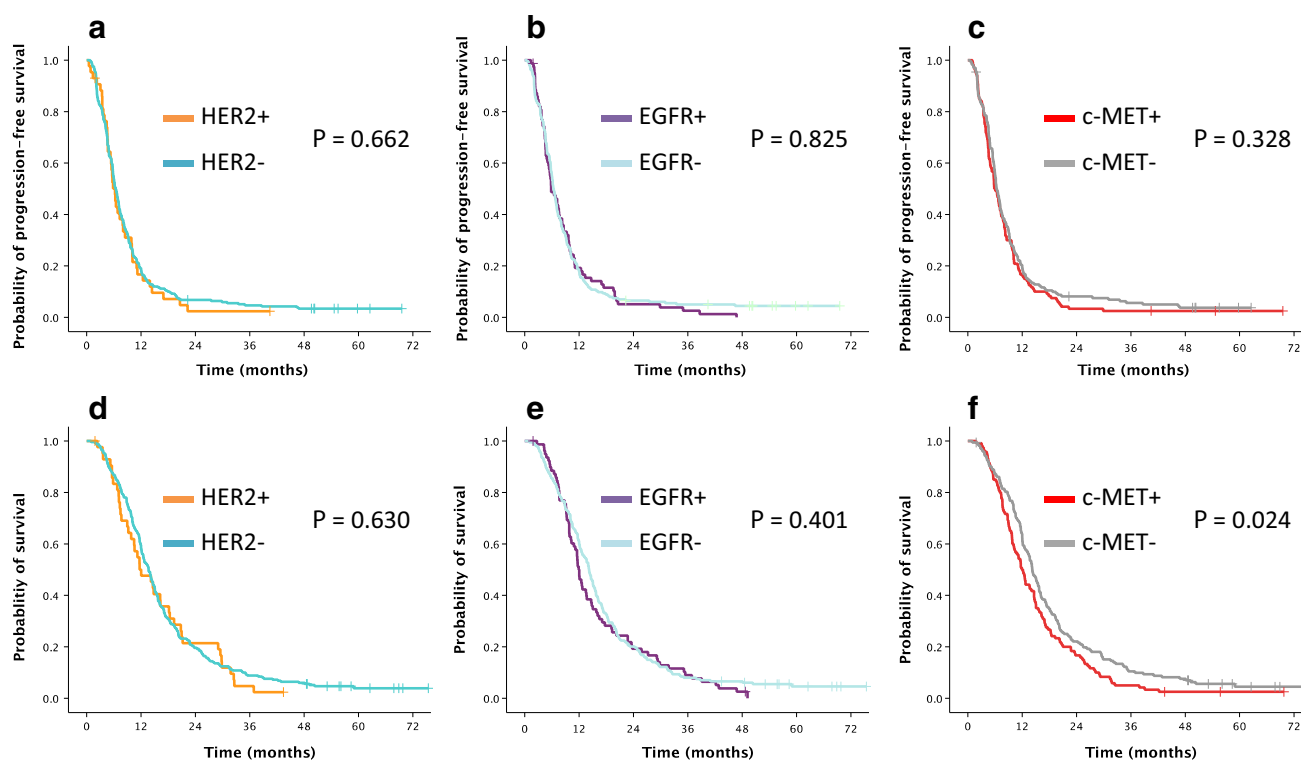
Fig. 2 Progression-free survival and overall survival stratified by human epidermal growth factor receptor 2 (*HER2*; **a**, **d**), epidermal growth factor receptor (*EGFR*; **b**, **e**), and c-MET (**c**, **f**). *P*, log-rank *P* value

characteristics were significantly different between c-MET-positive and c-MET-negative patients in terms of histological appearance ($P = 0.012$), liver metastasis ($P = 0.034$), and ALP value ($P = 0.047$; Table S1). Although there was no significant difference in PFS [median, 5.8 months (95 % CI, 4.9–6.8 months) vs 6.4 months (95 % CI, 5.7–7.1 months); HR, 1.13 (95 % CI, 0.89–1.43); log-rank $P = 0.328$; Fig. 2c], there was a significant difference in OS [median, 11.9 months (95 % CI, 10.7–13.2 months) vs 14.2 months (95 % CI, 12.9–15.5 months); HR, 1.31 (95 % CI, 1.03–1.67); log-rank $P = 0.024$; Fig. 2f] between c-MET-positive and c-MET-negative patients. The proportions of patients who received second-line and third-line chemotherapy were not significantly different between c-MET-positive and c-MET-negative patients [69 % vs 75 % for second-line chemotherapy ($P = 0.287$) and 38 % vs 47 % for third-line chemotherapy ($P = 0.153$)].

Co-overexpression of HER2, EGFR, and c-MET

Co-overexpression status of HER2, EGFR, and c-MET is shown in Fig. 3. Ten patients (3 %) exhibited simultaneous positive status for HER2, EGFR, and c-MET. In the ten simultaneous positive cases, some tumors have thr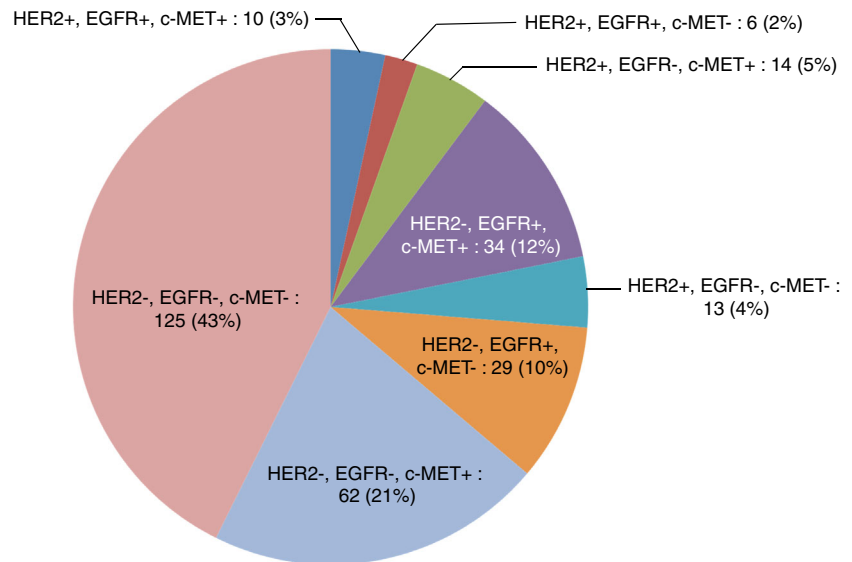ee independent areas that exhibited only one protein overexpression (or gene amplification) area, and some tumors have simultaneously two or three protein overexpression (or gene amplification) areas and only one protein overexpression (or gene amplification) area. No specific trends for this heterogeneity and co-overexpression were observed. There was no significant difference in OS among patients with co-overexpression status (data not shown). EGFR positivity between HER2-positive and HER2-negative patients was not significantly different (37% vs 25 %; $P = 0.135$). In contrast, c-MET positivity between HER2-positive and HER2-negative patients (56 % vs 38 %) and EGFR positivity between c-MET-positive and c-MET-negative patients (37 % vs 20 %) were significantly different ($P = 0.043$ and $P = 0.002$, respectively).

Multivariate analysis

The multivariate analysis without ALP revealed that c-MET positivity was still a significant prognostic factor for OS [HR, 1.30 (95 % CI, 1.02–1.67); $P = 0.037$; Table 3]. As a sensitivity analysis, we conducted a multivariate analysis involving 284 patients, because there was no baseline ALP value for nine patients. It also showed that c-MET positivity was a significant prognostic factor for OS [HR, 1.32 (95 % CI, 1.02–1.69); $P = 0.033$].

Fig. 3 Co-overexpression status of human epidermal growth factor receptor 2 (*HER2*), epidermal growth factor receptor (*EGFR*), and c-MET

HER2+, EGFR+, c-MET+ : 10 (3%)
HER2+, EGFR+, c-MET- : 6 (2%)
HER2+, EGFR-, c-MET+ : 14 (5%)
HER2-, EGFR+, c-MET+ : 34 (12%)
HER2+, EGFR-, c-MET- : 13 (4%)
HER2-, EGFR+, c-MET- : 29 (10%)
HER2-, EGFR-, c-MET- : 125 (43%)
HER2-, EGFR-, c-MET+ : 62 (21%)

Table 3 Results of multivariate analyses for overall survival ($n = 293$)

Variable	HR	95 % CI	P
ECOG PS, 0	0.78	0.60–1.02	0.067
Primary tumor site, stomach	1.47	0.98–2.21	0.061
Disease status, unresectable	0.52	0.32–0.85	*0.009*
Previous gastrectomy, yes	0.32	0.21–0.47	*≤0.001*
Previous adjuvant chemotherapy, yes	1.78	0.99–3.20	0.054
Histological appearance, differentiated	0.73	0.56–0.94	*0.014*
Measurable disease, yes	1.32	0.95–1.83	0.094
Liver metastasis	1.56	1.16–2.10	*0.004*
Peritoneal metastasis	1.27	0.96–1.69	0.098
c-MET, positive	1.30	1.02–1.67	*0.037*

Values in *italics* indicate statistical significance.

CI confidence interval, *ECOG PS* Eastern Cooperative Oncology Group performance status, *HR* hazard ratio

Discussion

To the best of our knowledge, this is the first study that has investigated co-overexpression of HER2, EGFR, and c-MET in AGC patients who received standard chemotherapy; we found that only c-MET was a significant and independent prognostic factor, which suggests that c-MET would be a good candidate for molecular target agents. There was no significant difference in terms of PFS for S-1 plus cisplatin. Although there were no statistically significant differences in the proportions of patients who received second-line and third-line chemotherapy, fewer c-MET-positive patients received second-line and third-line chemotherapy; this might have contributed to their poor prognosis. However, we could not reach a definitive conclusion as to the reason for their poor prognosis in this study. We observed c-MET overexpression more frequently in patients with disease with differentiated histological appearance, liver metastasis, and increased ALP levels, which was consistent with the findings of previous studies, except for the ALP levels [20, 24, 27].

Our observation that HER2 was not a prognostic factor in AGC patients was consistent with recent studies that evaluated HER2 using IHC combined with in situ hybridization [14, 32–34]. Although previous studies have reported that HER2 overexpression was more frequent in the GEJ than in the stomach [1], there was no significant difference in the incidence of HER2 positivity between the GEJ and the stomach in our study. The frequency of occurrence of GEJ tumors in our study was relatively small (10 %) compared with those reported in recent global clinical trials (16–29 %) [4, 35, 36]. Although the exact reason for the discrepancy with other studies remains unknown, our study results were consistent with a recent report concerning Japanese patients: the presence of HER2 overexpression was not influenced by tumor location [37].

Although EGFR has been reported to be a prognostic factor, findings from our study were not consistent with those from previous studies [11–14]. The primary anti-EGFR antibody used and the diagnostic criteria were different among the studies. Most importantly, there was a difference in the target populations. Most of the former studies mainly consisted of patients with resectable disease, whereas our study was conducted in patients with unresectable or recurrent disease. Our analysis was consistent with the recent biomarker analysis from the large clinical trial of AGC patients that reported no relationship between the EGFR IHC score and prognosis [4].

In the present study, approximately 60 % of AGC patients have overexpression of one or more receptors that can be a target of molecular targeted therapy. Although trastuzumab combined with chemotherapy is a standard first-line treatment for HER2-positive AGC patients, no target-based standard therapy has been established for the remaining AGC patients. For EGFR, although the clinical trials evaluating the anti-EGFR antibodies, such as cetuximab or panitumumab combined with chemotherapy as first-line treatment, failed to show any benefits in non-selected AGC patients, the phase III trial of nimotuzumab as a second-line treatment for EGFR-positive patients is ongoing. For c-MET and its ligand, hepatocyte growth factor, phase III trials of onartuzumab and rilotumumab in AGC patients with c-MET overexpression and a considerable number of phase I trials of c-MET-targeting agents are ongoing [5]. Therefore, a new treatment strategy for patients with simultaneous positivity for EGFR or c-MET and HER2 is required [25].

Our study had several limitations. First, the diagnostic criteria for EGFR and c-MET status were tentative and not standardized. Second, we analyzed EGFR and c-MET only for protein overexpression using IHC, and not for gene amplification. In terms of clinical utility, standardized methods and diagnostic criteria should be established on the basis of the ability to evaluate pharmacological response to therapeutic intervention. Although an exploratory analysis of the clinical utility of EGFR and c-MET status has been conducted in recent clinical trials [4, 5], their diagnostic criteria have not been validated. Therefore, diagnostic criteria regarding EGFR and c-MET should be investigated in the ongoing or future prospective clinical trials. Since recent clinical trials often use the diagnostic criteria with IHC for gastric cancer patient enrichment, the correlation between IHC overexpression and the affinity of each drug, and tumor heterogeneity may define the success of clinical development of each agent.

Conclusions

Findings from the present study suggest that HER2 and EGFR status had no significant prognostic impact in terms of OS in AGC patients treated with conventional chemotherapy as a first-line treatment. In addition, c-MET-positive AGC patients had a poorer prognosis than c-MET-negative patients. These data could be used as the basis for future clinical trials for targeting agents in the treatment of AGC patients.

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