


Clinical impact of intratumoral HER2 heterogeneity on trastuzumab efficacy in patients with HER2-positive gastric cancer

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Abstract

Background There is growing interest in the clinical significance of intratumoral HER2 heterogeneity. Its prognostic and predictive impacts on trastuzumab efficacy were demonstrated in breast cancer. However, its clinical significance in gastric cancer is still unclear.

Methods Twenty-eight HER2-positive gastric cancer patients who had gastrectomy prior to trastuzumab-based chemotherapy were consecutively enrolled. Intratumoral HER heterogeneity was evaluated using whole-tissue sections by immunohistochemistry. When all tumor cells overexpressed HER2 protein, the tumor was defined as homogeneously HER2 (Homo-HER2)-positive group. The others were defined as heterogeneously HER2 (Hetero-HER2)-positive group.

Results There was no significant difference in clinicopathological features between the two groups. The median progression-free survival (PFS) and overall survival (OS) in the Homo-HER2-positive group were significantly longer than those in the Hetero-HER2-positive group (PFS; 20.0 months [95% CI 17.8–22.2] vs. 6.0 months [95% CI

2.3–9.7]; HR 0.11; 95% CI 0.03–0.41; $p < 0.001$, OS; not reached vs. 14.0 months [95% CI 11.9–16.1]; HR 0.18; 95% CI 0.06–0.61; $p = 0.003$). In the multivariate analysis, these associations remained significant both in PFS (HR 0.12; 95% CI 0.03–0.46, $p = 0.002$) and OS (HR 0.21; 95% CI 0.06–0.72, $p = 0.013$). With respect to response rate, no statistical difference was found between two groups. However, deeper tumor shrinkage was obtained in the Homo-HER2-positive group compared with the Hetero-HER2-positive group ($p = 0.046$).

Conclusions Intratumoral HER2 heterogeneity may have robust clinical impact on trastuzumab efficacy in patients with HER2-positive gastric cancer. These findings should be validated by larger independent cohorts and further molecular correlative analyses are warranted.

Keywords Intratumoral HER2 heterogeneity · Trastuzumab · Predictive marker · Chemotherapy · Gastric cancer

Abbreviations

CEP17	Centromeric probe 17
FISH	Fluorescence in situ hybridization
HER2	Human epidermal growth factor receptor 2
Homo-HER2 positive	Homogeneously HER2 positive
Hetero-HER2 positive	Heterogeneously HER2 positive
IHC	Immunohistochemistry
PFS	Progression-free survival
ORR	Objective response rates
OS	Overall survival
RTK	Receptor tyrosine kinase

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Introduction

Gastric cancer is currently the third-most common cause of cancer-related deaths and the fifth-most common diagnosed cancer worldwide [1]. Endoscopic resection, standard gastrectomy with D2 lymphadenectomy, and multidisciplinary therapy have improved outcomes [2–5]. However, the prognosis for inoperable or metastatic gastric cancer is still poor and median overall survival (mOS) is less than 1 year globally [6, 7].

Human epidermal growth factor receptor 2 (HER2) is involved in tumor proliferation, differentiation, and survival [8]. Its amplification or protein overexpression is shown in up to 20% of cases in gastric cancer [9–11], suggesting that it is a critical driver oncogene and, therefore, a promising treatment target. Trastuzumab, full humanized anti-HER2 antibody, has been successfully developed in HER2-positive breast cancer [12, 13]. In gastric cancer, the ToGA trial showed significant clinical benefits of the addition of trastuzumab to standard chemotherapy [14]. Inclusion criteria of the ToGA trial were initially designed as HER2 immunohistochemistry (IHC) 3+ or fluorescence in situ hybridization (FISH) positive. But post hoc analysis showed that HER2 IHC 3+ or IHC 2+/FISH-positive groups benefited more from trastuzumab than the other groups, leading to different indications for trastuzumab use across the world. In the US, trastuzumab was approved for IHC 3+ or FISH positive. Meanwhile, in Europe and Japan, trastuzumab use is limited in IHC 3+ or IHC 2+/FISH positive.

Trastuzumab-based first-line treatment now represents a standard approach. However, its treatment efficacy varies among patients; the overall response rate is widely reported with frequency from 47.0 to 68.0% [14–16]. This indicates that there is a considerable proportion of patients refractory to trastuzumab and the present criteria for trastuzumab use may be insufficient to predict the response to trastuzumab. Therefore, a new biomarker which can predict trastuzumab efficacy is needed.

There is growing interest in intratumoral HER2 heterogeneity in breast and gastric cancer. Compared with breast cancer, HER2 heterogeneity in gastric cancer has been frequently recognized from 39.0 to 75.4% evaluated by IHC [17–21] and occasionally becomes a problem for assessment of HER2 [22]. Clinical significance of intratumoral HER2 heterogeneity as a predictive factor for trastuzumab was reported in breast cancer; namely, homogeneously HER2 overexpressed breast cancer showed more benefit from trastuzumab-based treatment compared with heterogeneously HER2 overexpressed breast cancer [23]. However, its clinical significance in gastric cancer is still unclear [24].

We hypothesized that, as in HER2-positive breast cancer, intratumoral HER2 heterogeneity may have clinical impact on trastuzumab efficacy in HER2-positive gastric cancer. To test our hypothesis, we evaluated intratumoral HER2 heterogeneity within a tumor and investigated its clinical relevance to trastuzumab efficacy in patients with HER2-positive advanced gastric cancer.

Methods

Patients

One-hundred and thirty-one patients with histologically confirmed HER2-positive advanced or metastatic gastric cancer were treated by trastuzumab-based chemotherapy as first-line treatment in The Cancer Institute Hospital of Japanese Foundation for Cancer Research from March 2011 to December 2015. Among them, 28 patients, who had previous gastrectomy and whose primary tumor samples were available, were consecutively enrolled to evaluate intratumoral HER2 heterogeneity in the whole-tissue sections. Computed tomography assessments were repeated every 6–8 weeks and RECIST ver1.1 was used to define all responses. Clinical information was retrospectively collected from medical records. This study was approved by the Institutional Review Boards (No. 2015-1029) and all patients signed an informed consent for the analysis of molecular correlates.

Treatment schedule

Chemotherapy was done in accordance with the ToGA trial [14]. Briefly, cisplatin 80 mg/m² on day 1 was given by intravenous infusion and it was given every 3 weeks until six cycles. Capecitabine 1000 mg/m² was given orally twice a day for 14 days followed by a 1-week rest. Trastuzumab was given by intravenous infusion at a dose of 8 mg/kg on day 1 of the first cycle, followed by 6 mg/kg every 3 weeks. Treatments were continued until disease progression or patient's refusal or intolerable adverse effect despite appropriate dose reduction.

Evaluation of HER2

A formalin-fixed paraffin-embedded block including the maximum invasive area of the tumor was selected in each patient and was cut into 4- μ m section. IHC was conducted using an BenchMark ULTRA autostainer (Ventana, Tucson, AZ, USA) and the primary antibody used was anti-HER2 (4B5) (10798; rabbit monoclonal; Ventana, Tucson, AZ, USA) according to the manufacturer's instructions. The HER2 protein overexpression was evaluated using the

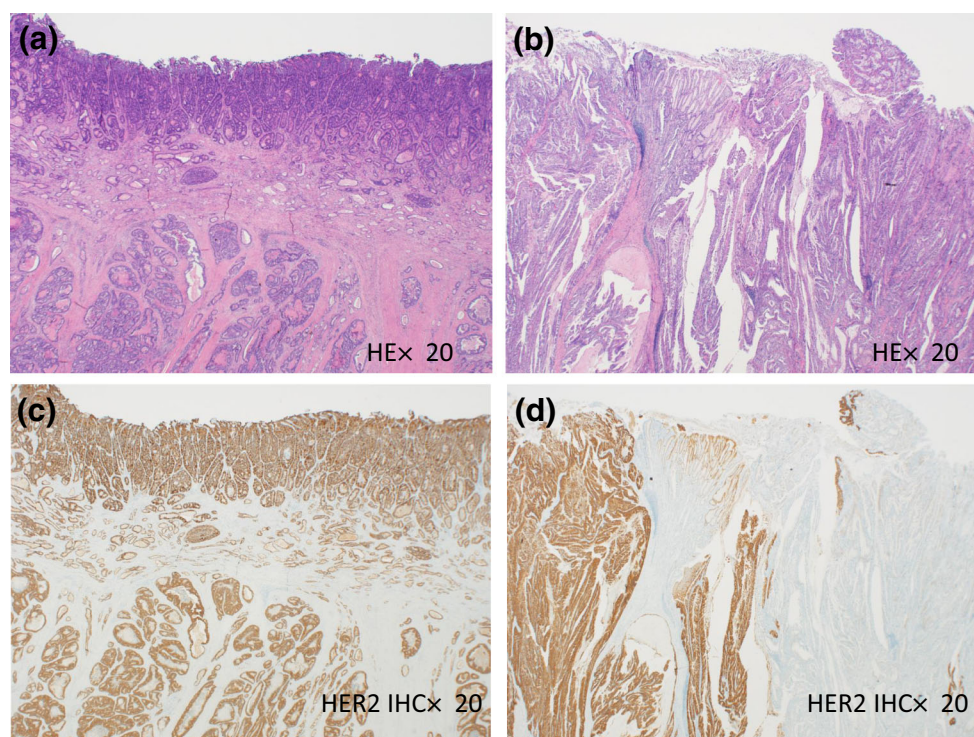


Fig. 1 Representative images of homogeneously HER2 positive (c) and heterogeneously HER2 positive (d) in gastric cancer. Hematoxylin–eosin stain shows differentiated adenocarcinoma in **a** and **B** ($\times 20$). HER2 IHC shows that almost all cancer cells

overexpress HER2 protein in **c** ($\times 20$). HER2 IHC shows heterogeneously HER2 overexpression: clearly demarcated HER2-positive area is recognized in left side while negative in right side in **d** ($\times 20$)

ToGA trial scoring system for surgical resected gastric cancer tissue obtained from primary lesion: 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 [14]. When IHC score was 2+ by IHC, FISH was performed using Path Vysion HER2 DNA probe kit (Vysis/Abbott, Abbott Park, IL, USA) according to the manufacturer's instructions. Briefly, the total numbers of HER2 and centromeric probe 17 (CEP17) signals were counted in at least 20 cancer cell nuclei in different two areas. When a HER2/CEP17 ratio was ≥ 2.2 , it was diagnosed as HER2 gene amplification. IHC score of 3+ or IHC score of 2+ with FISH positivity was defined as HER2 positive, whereas IHC score of 0 or 1+, or IHC score of 2+ with FISH negativity was defined as HER2 negative.

Evaluation of intratumoral HER2 heterogeneity

Intratumoral HER2 heterogeneity was independently evaluated by two pathologists (NY and HK) without any clinical information. Because there is no guideline for the

assessment of intratumoral HER2 heterogeneity in gastric cancer, we defined intratumoral HER2 heterogeneity as follows in this study; when all tumor cells on a section representing the tumor overexpressed HER2 protein with IHC 3+ or 2+, the tumor was defined as homogeneously HER2 (Homo-HER2)-positive group. The others were defined as heterogeneously HER2 (Hetero-HER2)-positive group. Representative images are shown in Fig. 1.

Statistical analysis

Progression-free survival (PFS), OS, and objective response rates (ORRs) were evaluated. PFS was defined between the date of initial chemotherapy and first documented progression or death from any cause. OS was defined between the date of initial chemotherapy and death from any cause. If patients did not meet any endpoints until 31 December 2016, they were censored at the time of last contact. To evaluate the prognostic value of intratumoral HER2 heterogeneity, PFS and OS were estimated using Kaplan–Meier methods and compared by the log-rank test. Multivariate analysis using Cox proportional hazard model was performed by including covariates whose *p* value was less than 0.05 in univariate analysis. Patient characteristics and response rates were evaluated by Fisher's exact test.

Depth of response was compared using Mann–Whitney *U* test. The level of significance was set to $p < 0.05$ and all statistical tests were two sided. All statistical analysis was done using the SPSS ver.24 (SPSS Chicago, IL).

Results

Patient characteristics

Patient characteristics in this study are shown in Table S1. Male gender, stomach location, and differentiated

histology were dominant in this cohort. More than half of patients had visceral (lung or liver) metastasis. Majority had received adjuvant chemotherapy using S-1, an oral fluoropyrimidine, for 1 year after the previous curative surgery [3]. None had any neoadjuvant chemotherapy prior to surgery. More than 90% of the patients were IHC 3+. Intratumoral HER2 heterogeneity was observed in half of the patients (50.0%). Concordance rate of diagnosis of HER2 heterogeneity between the two pathologists was 100%. Twenty-five patients were treated with capecitabine and cisplatin plus trastuzumab, while three patients were similarly treated without cisplatin due to old age. Table 1

Table 1 Comparison of patients’ characteristics between Homo-HER2 and Hetero-HER2-positive groups in this study

Characteristics	Hetero-HER2 <i>n</i> = 14 (%)	Homo-HER2 <i>n</i> = 14 (%)	<i>p</i> values
Age	68.5	68.0	1.000
≥ 68	8 (57.1)	7 (50.0)	
< 68	6 (42.9)	7 (50.0)	
Gender			0.385
Male	12 (85.7)	9 (64.3)	
Female	2 (14.3)	5 (35.7)	
ECOG PS			1.000
0	8 (57.1)	8 (57.1)	
1–2	6 (42.9)	6 (42.9)	
Primary tumor site			1.000
GEJ	4 (28.6)	4 (28.6)	
Distal stomach	10 (71.4)	10 (71.4)	
Differentiation			0.326
Differentiated type	13 (92.9)	10 (71.4)	
Undifferentiated type	1 (7.1)	4 (28.6)	
Visceral metastasis			1.000
Yes	8 (57.1)	7 (50.0)	
No	6 (42.9)	7 (50.0)	
Adjuvant chemotherapy			1.000
Yes	8 (57.1)	9 (64.3)	
No	6 (42.9)	5 (35.7)	
Platinum-based			0.222
Yes	11 (78.6)	14 (100)	
No	3 (21.4)	0 (0.0)	
HER2 status			0.481
IHC 3+	12 (85.7)	14 (100)	
IHC 2+/FISH positive	2 (14.3)	0 (0.0)	
CEA			0.449
≥ 5.0 ng/ml	9 (64.3)	6 (42.9)	
< 5.0 ng/ml	5 (35.7)	8 (57.1)	
CA 19-9			1.000
≥ 37.0 U/ml	7 (50.0)	6 (42.9)	
< 37.0 U/ml	7 (50.0)	8 (57.1)	

Hetero-HER2 heterogeneously HER2 positive, *Homo-HER2* homogeneously HER2 positive, *GEJ* gastroesophageal junction, *ECOG PS* Eastern Cooperative Oncology Group Performance Status, *FISH* fluorescence in situ hybridisation, *IHC* immunohistochemistry, *CEA* carcinoembryonic antigen, *CA 19-9* carbohydrate antigen 19-9

shows a comparison of demographics between the two groups. The patients who were treated without cisplatin and the patients whose tumor was IHC 2+/FISH positive were all found in the Hetero-HER2-positive group; however, there was no significant difference in all characteristics between the two groups.

Clinical impact of intratumoral HER2 heterogeneity on trastuzumab efficacy

The median follow-up period was 17.9 months for all patients and 33.0 months for the censored patients, respectively. The median PFS and OS were 11.0 months (95% CI 7.1–14.9) and 24.0 months (95% CI 4.6–43.4), respectively. Twenty-five patients (89.3%) discontinued the trastuzumab-based chemotherapy due to disease progression, whereas three patients were still on treatment. These three patients belonged to the Homo-HER2-positive group. The median number of cycles of trastuzumab therapy was 23.7 (range 2–62) in the Homo-HER2-positive group and 9.3 (range 1–18) in the Hetero-HER2-positive group, respectively. Fifteen patients (53.6%) died at the data cutoff. Second-line chemotherapy after disease progression was given to all patients (11/11) in the Homo-HER2-positive group. Meanwhile, in the Hetero-HER2-positive group, 11 of 14 (78.6%) patients received second-line chemotherapy. Taxans as monotherapy or weekly paclitaxel with ramucirumab were mainly initiated as post-discontinuation therapy in the both groups.

Survival is shown in Fig. 2. The median PFS in the Homo-HER2-positive group was significantly longer than that in the Hetero-HER2-positive group (20.0 months [95% CI 17.8–22.2] vs. 6.0 months [95% CI 2.3–9.7]; HR 0.11; 95% CI 0.03–0.41; $p < 0.001$;

95% CI 0.03–0.41; $p < 0.001$; Fig. 2a). The median OS in the Homo-HER2-positive group was also significantly longer than that in the Hetero-HER2-positive group (not reached vs. 14.0 months [95% CI 11.9–16.1]; HR 0.18; 95% CI 0.06–0.61; $p = 0.003$; Fig. 2b). Platinum-based chemotherapy was also associated with both in PFS and OS in this cohort (Table 2). In the multivariate analysis stratified by HER2 heterogeneity and chemotherapy regimen, intratumoral HER2 heterogeneity remained significant both in PFS and OS (Table 3). In addition, because of small sample size, multivariate analyses were performed stratified by intratumoral HER2 heterogeneity and each single covariate chosen from all covariates. Intratumoral HER2 heterogeneity was the only independent predictor for PFS and OS (data not shown).

Because our data showed shorter survival of Hetero-HER2-positive group, it is unclear whether patients with Hetero-HER2-positive gastric cancer benefit from additional trastuzumab. Next, we compared survival of the Hetero-HER2-positive group and those of HER2-negative gastric cancer who received cisplatin and capecitabine. Eighteen patients with HER2-negative gastric cancer received cisplatin and capecitabine in the same period. The median PFS and OS were 5.7 months (95% CI 0.0–11.4) and 14.1 months (95% CI 6.6–21.6), respectively. No statistical difference was found between the Hetero-HER2-positive and HER2-negative group (HR 1.21; 95% CI 0.59–2.50; $p = 0.598$ for PFS and HR 0.97; 95% CI 0.44–2.13; $p = 0.931$ for OS), respectively, although the sample number is small (Fig S1).

Twenty patients had measurable lesions and best overall responses in these patients by RECIST were complete response in 5 patients (25%), partial response in 6 patients

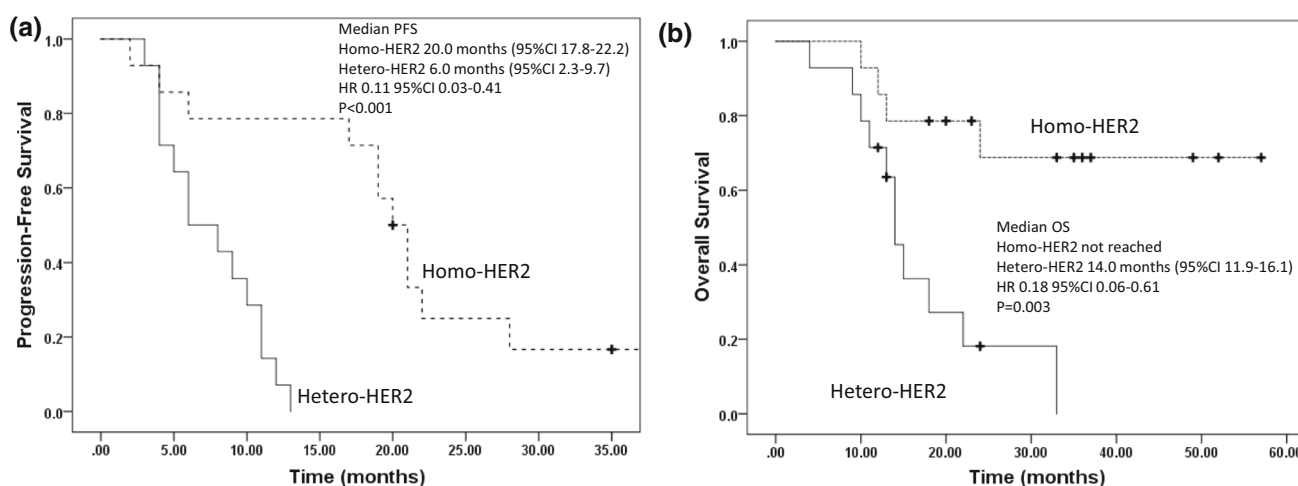


Fig. 2 Progression-free survival and overall survival. Significant longer progression-free survival was seen in the Homo-HER2-positive group compared with the Hetero-HER2-positive group (a).

Significant longer overall survival was seen in the Homo-HER2-positive group compared with the Hetero-HER2-positive group (b)

Table 2 Survival by univariate analysis

Characteristics (n)	mPFS (95% CI)	HR (95% CI)	p values	mOS (95% CI)	HR (95% CI)	p values
Median age (years)			0.373			0.124
≥ 68 (15)	9.0 (3.3–14.7)	1 (Reference)		18.0 (9.2–26.8)	1 (Reference)	
< 68 (13)	17.0 (9.2–24.8)	0.71 (0.32–1.56)		NR	0.44 (0.15–1.30)	
Gender			0.143			0.323
Male (21)	10.0 (5.5–14.5)	1 (Reference)		22.0 (7.5–36.5)	1 (Reference)	
Female (7)	19.0 (0.0–39.5)	0.49 (0.18–1.33)		NR	0.48 (0.11–2.14)	
ECOG PS			0.865			0.936
0 (16)	11.0 (5.1–16.9)	1 (Reference)		24.0 (4.7–43.3)	1 (Reference)	
1–2 (12)	10.0 (1.5–18.5)	1.07 (0.48–2.39)		22.0 (3.6–40.4)	1.04 (0.37–2.94)	
Tumor site			0.750			0.655
GEJ (8)	13.0 (6.1–19.9)	1 (Reference)		33.0 (4.1–61.9)	1 (Reference)	
Stomach (20)	9.0 (0.2–17.8)	1.15 (0.48–2.73)		22.0 (9.6–34.4)	1.25 (0.41–4.08)	
Differentiation			0.737			0.550
Differentiated type (23)	11.0 (7.9–14.1)	1 (Reference)		24.0 (4.3–43.7)	1 (Reference)	
Undifferentiated type (5)	9.0 (2.6–15.4)	1.20 (0.40–3.58)		15.0 (10.7–19.3)	1.48 (0.40–5.48)	
Visceral metastasis			0.116			0.271
Yes (15)	8.0 (3.3–12.7)	1 (Reference)		15.0 (11.5–18.5)	1 (Reference)	
No (13)	13.0 (3.6–22.4)	0.54 (0.24–1.21)		33.0 (NA)	0.56 (0.20–1.60)	
Adjuvant chemotherapy			0.856			0.702
Yes (17)	11.0 (5.6–16.4)	1 (Reference)		33.0 (0.6–65.4)	1 (Reference)	
No (11)	10.0 (0.0–24.0)	0.93 (0.42–2.08)		22.0 (11.2–32.8)	1.22 (0.44–3.37)	
Platinum-based			0.012			0.025
Yes (25)	12.0 (8.3–15.7)	1 (Reference)		24.0 (5.1–42.9)	1 (Reference)	
No (3)	4.0 (NA)	4.69 (1.18–18.65)		11.0 (7.8–14.2)	5.64 (1.02–31.38)	
HER2 status			0.283			0.273
IHC 3+ (26)	11.0 (6.0–16.0)	1 (Reference)		22.0 (9.4–34.6)	1 (Reference)	
IHC 2+/FISH positive (2)	5.0 (NA)	2.17 (0.49–9.63)		NR	0.04 (0.0–251.4)	
HER2 heterogeneity			<0.001			0.003
Yes (14)	6.0 (2.3–9.7)	1 (Reference)		14.0 (11.9–16.1)	1 (Reference)	
No (14)	20.0 (17.8–22.2)	0.11 (0.03–0.41)		NR	0.18 (0.06–0.61)	
CEA			0.659			0.804
≥ 5.0 ng/ml (15)	6.0 (0.0–12.3)	1 (Reference)		22.0 (8.6–35.4)	1 (Reference)	
< 5.0 ng/ml (13)	12.0 (5.0–19.1)	0.84 (0.38–1.85)		24.0 (1.2–46.9)	0.88 (0.32–2.43)	
CA 19-9			0.627			0.641
≥ 37.0 U/ml (13)	8.0 (2.4–13.6)	1 (Reference)		18.0 (9.9–26.1)	1 (Reference)	
< 37.0 U/ml (15)	11.0 (2.2–19.8)	0.82 (0.37–1.81)		24.0 (NA)	0.64 (0.23–1.77)	

GEJ gastroesophageal junction cancer, ECOG PS Eastern Cooperative Oncology Group Performance Status, HR hazard ratio, 95% CI 95% confidential interval, mPFS median progression-free survival, mOS median overall survival, NA not applicable, NR not reached, CEA carcinoembryonic antigen, CA 19-9 carbohydrate antigen 19-9

(30%), stable disease in 8 patients (40%), and progression disease in 1 patients (5%). ORR was 55.0% and disease control rate was 95.0%. A higher response rate was seen in the Homo-HER2-positive group than in the Hetero-HER2-positive group (77.8 vs. 36.4%; $p = 0.092$; Table 4). Moreover, deeper response was seen in the Homo-HER2-positive group compared with the Hetero-HER2-positive group ($p = 0.046$; Fig. 3).

Comparison of HER2 heterogeneity between surgical and biopsy specimens

Since the majority of patients who receive trastuzumab-based chemotherapy are unresectable, we examined whether biopsy specimens are useful to determine HER2 heterogeneity and to predict treatment efficacy. With respect to the definition of HER2 heterogeneity, the same as surgical specimen, if all tumor cells overexpressed

Table 3 Survival outcomes by multivariate analysis

Covariates	PFS		OS	
	HR 95% (CI)	<i>p</i> values	HR 95% (CI)	<i>p</i> values
Platinum-based				
Yes (25)	1 (Reference)	0.232	1 (Reference)	0.207
No (3)	2.34 (0.58–9.37)		3.06 (0.54–17.39)	
HER2 heterogeneity				
Yes (14)	1 (Reference)	0.002	1 (Reference)	0.013
No (14)	0.12 (0.03–0.46)		0.21 (0.06–0.72)	

HR hazard ratio, 95% CI 95% confidential interval, PFS progression-free survival, OS overall survival

Table 4 Best overall response

	Hetero-HER2 (%) <i>n</i> = 12	Homo-HER2 (%) <i>n</i> = 8	<i>p</i> values
CR	1 (8.3)	2 (25.0)	
PR	5 (41.7)	5 (62.5)	
SD	6 (50.0)	0 (0)	
PD	0 (0)	1 (12.5)	
ORR	6/12 (50.0)	7/8 (87.5)	0.282

CR complete response, PR partial response, SD stable disease, PD progression disease, ORR objective response rate, Hetero-HER2 heterogeneously HER2 positive, Homo-HER2 homogeneously HER2 positive

HER2 protein with IHC 3+ or 2+ on all biopsy specimens, the tumor was defined as the Homo-HER2-positive group. The others were defined as the Hetero-HER2-positive group. Biopsy specimens were available for 25 patients (89%). Two of them were HER2 negative by examination

of biopsy specimens, and these two patients belonged to the Hetero-HER2-positive group determined by surgical specimens. Comparison of HER2 heterogeneity between biopsy and surgical specimens is shown in Table S2. All Hetero-HER2-positive patients determined by surgical specimens were judged as Hetero-HER2 positive by biopsy specimens, whereas 2 of 12 patients determined as Homo-HER2 positive by surgical specimens were judged as Hetero-HER2 positive by biopsy specimens. Concordance rate of HER2 heterogeneity between surgical and biopsy specimens was 92%. Sensitivity, specificity, positive predict value, and negative predict value of evaluation biopsy specimens were 100, 83.3, 100, and 86.7%, respectively. With respect to survival, similar results to surgical specimens were shown: Homo-HER2-positive group determined by biopsy specimens showed longer PFS (20.0 months [95% CI 17.9–22.1] vs. 8.0 months [95% CI 4.2–11.8]; HR 0.18; 95% CI 0.06–0.54; *p* = 0.002) and OS (not reached

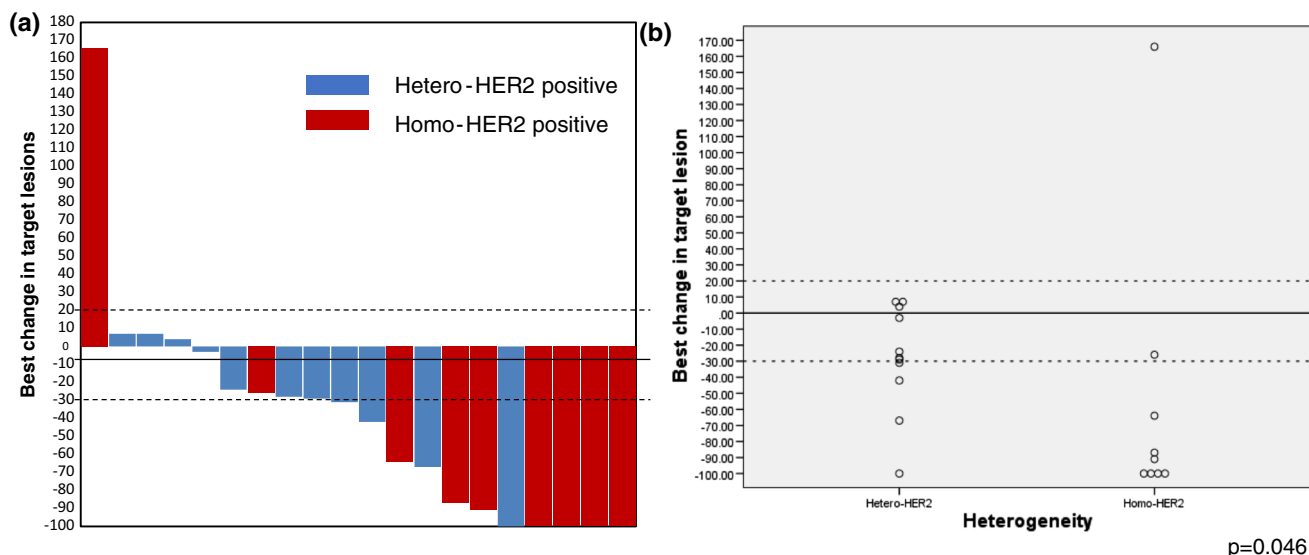


Fig. 3 Best change from baseline in size of target lesion. Water-fall plot shows that patients in the Homo-HER2-positive group obtain deeper tumor shrinkage compared with the Hetero-HER2-positive

group (a). Scatter plot shows statistically significant difference in tumor shrinkage between two groups (*p* = 0.046) (b)

vs. 14.0 months [95% CI 12.4–15.6]; HR 0.09; 95% CI 0.02–0.44; $p = 0.003$; Fig S2).

Discussion

In this study, we carefully evaluated intratumoral HER2 heterogeneity using surgical specimens and examined predictive impact on trastuzumab efficacy. Our results showed, similar to breast cancer, patients with homogeneously HER2-positive gastric cancer had significantly longer survival than those with heterogeneously HER2-positive gastric cancer. In addition, higher response rate and deeper tumor shrinkage were found in the Homo-HER2-positive group. To our knowledge, this is the first report describing the clinical impact of intratumoral HER2 heterogeneity examined in surgical specimens on trastuzumab efficacy in patients with HER2-positive gastric cancer.

There have been several reports regarding intratumoral HER2 heterogeneity in gastric cancer [17–21]. According to these reports, HER2 heterogeneity was associated with IHC 2+ and diffuse histology. The incidences of intratumoral HER2 heterogeneity evaluated by IHC have been widely reported with a frequency from 39.0 to 75.4%. Kurokawa et al. reported the highest frequency of HER2 heterogeneity of 75.4% using whole-tissue sections when a cutoff value was set at 90% [18]. Meanwhile, screening data from the ToGA study, in which approximately 70% of the samples were biopsy specimens, showed that HER2 heterogeneity was found in 50.3% of the patients when the cutoff value was set at 30% [21]. Various cutoff values for HER2 heterogeneity assessment and different types of samples could affect the widely different frequencies of intratumoral HER2 heterogeneity in gastric cancer. In this study, we examined whole-layer surgical specimens and set the cutoff value as 100% in a representative section. In addition, our data demonstrated not only higher concordance rate of HER2 heterogeneity between surgical and biopsy specimens but similar predictive impact to surgical specimens, suggesting the utility of predictive impact of HER2 heterogeneity using biopsy specimen in unresectable HER2-positive gastric cancer.

In this study, patients in the Homo-HER2-positive group obtained greater benefit from trastuzumab-based chemotherapy compared with those in the Hetero-HER2 group. Previous data showed that homogeneous HER2 protein overexpression was associated with higher gene amplification by FISH [17]. In addition, recent reports demonstrated that higher *HER2* amplification level was associated with favorable trastuzumab efficacy [25, 26]. These reports are compatible with our survival results. Moreover, comprehensive genomic alternation and protein

overexpression analysis revealed that cases with high-level receptor tyrosine kinase (RTK) amplification showed simple tumor biology such as corresponding protein overexpression and, rarely, other gene amplification [27]. Therefore, homogeneous HER2-positive gastric cancer may link to simple tumor biology, which means that almost all tumor cells depend on the HER2-driven pathway and these patients are probably the optimal population for anti-HER2 agent treatment. On the other hand, patients in the Hetero-HER2-positive group did not obtain enough survival benefit from trastuzumab. Previous data demonstrated that HER2 heterogeneity was involved in co-amplification or co-overexpression of other RTKs such as EGFR, cMET, and FGFR2 [27–30]. These molecular diversities may result in trastuzumab resistance, suggesting the requirement of new treatment strategy. Multikinase inhibitors or combination treatment corresponding to molecular alternations possibly overcome molecular diversity in gastric cancer [31, 32].

The mechanism of HER2 heterogeneity remains unclear. Lee et al. examined HER2 heterogeneity individually by IHC and FISH in the same area, and they revealed a strong correlation between HER2 protein over-expression and gene amplification. These data suggest that genetic heterogeneity is underlying in HER2 heterogeneity [17]. In addition, several data showed that HER2 heterogeneity was associated with diffuse histology. Recent data from the cancer genome atlas proposed that gastric cancer was classified into four subtypes: Epstein–Barr virus positive, microsatellite unstable, chromosomal instability, and genomically stable tumor. Genomically stable tumors were enriched for diffuse histology and were associated with less frequency of RTKs amplification [30]. Because gastric cancer commonly showed histological heterogeneity, this histological heterogeneity may be related to genetic heterogeneity. Further molecular analysis in order to elucidate genetic heterogeneity in gastric cancer is warranted.

Unlike in breast cancer, the prognostic impact of HER2 is still controversial in gastric cancer [9, 18]. In addition, HER2 targeted agents, such as trastuzumab, lapatinib, T-DM1, and pertuzumab, have all been successfully developed in breast cancer, while all these anti-HER2 agents, except for trastuzumab, failed to show clinical benefit in gastric cancer [33–36]. Intratumoral HER2 heterogeneity may have influenced the inconsistencies in these results between breast and gastric cancers despite their having the same HER2 property: the median proportion with *HER2* amplification by FISH was 87.8% in breast cancer, whereas, in gastric cancer, almost half of the patients with HER2 protein overexpression exhibited it in less than 30% of the tumor cells [21, 23]. For future clinical trials for anti-HER2 agents in gastric cancer, consideration should be given not only to whether a tumor is HER2

positive but also to the degree in which the tumor depends on the HER2-driven pathway.

Limitations of this study include its retrospective nature from a single institution. In addition, the sample size is too small to conclude a clinical significance and the optimal cut-off value for trastuzumab. Nevertheless, our results would give credence and set a precedent for future research. In conclusion, intratumoral HER2 heterogeneity showed a clinical impact on trastuzumab efficacy in gastric cancer. These findings should be validated by larger independent cohorts and further molecular correlative analyses are warranted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures followed were in accordance with the Ethical Standards of the Responsible Committee on Human Experimentation (Institutional and National) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

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