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Research Article

The Clinical Significance of Immunohistochemistry-Heterogeneously HER2 Positive and Lauren Classification Combination in Gastric Cancer: A Meta-Analysis

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Abstract

Aim: To assess clinical significance of intratumoral heterogeneously Human Epidermal Growth Factor receptor 2 (HER2) positive and Lauren Classification (LC) combination for HER2-positive Gastric Cancer (GC).

Methods: Published studies investigating the effect of immunohistochemistry HER2 overexpression on GC with LC were identified. The patients were classified into 4 groups according to intratumoral HER2 overexpression status and LC types: heterog-HER2–LC intestinal type (heterog-HER2-LCI) group, homo-HER2–LC intestinal type (homo-HER2-LCI) group, hetero-HER2–LC diffuse type (heterog-HER2-LCD) group and homo-HER2–LC diffuse type (homo-HER2-LCD) group. Their Odds Ratio (OR) were compared. The OR and their corresponding 95% confidence intervals (95% CIs) were analyzed.

Results: 487 HER2 positive samples were included in the meta-analysis. There were 70% samples of heterog-HER2-LCI and 79% of homo-HER2-LCI, suggesting that homo-HER2-LCI rate was significantly higher than heterog-HER2-LCI in GC LC intestinal type patients (OR =0.53, 95% CI: 0.23-1.47, p=0.01). There were 30% samples of heterog-HER2-LCD and 20% of homo-HER2-LCD, suggesting that heterog-HER2-LCD rate was significantly higher than homo-HER2-LCD in GC LC intestinal type patients (OR=1.94, 95% CI: 1.20-3.14, p=0.007). For HER2-IHC (2+) analysis: the hetero-HER2 was observed in 32% samples and homo-HER2 was detected in 7% samples, revealing that the hetero-HER2+ rate was significantly higher than home-HER2+ in the GC HER2-IHC (2+) (OR=11.95, 95% CI: 5.57-25.64, p<0.00001). For staining intensity analysis of HER2-IHC (3+): the hetero-HER2 was observed in 68% samples and homo-HER2 was seen in 93% samples, indicating that hetero-HER2 rate was significantly lower than home-HER2 in the GC HER2-IHC (3+) samples (OR=0.08, 95% CI: 0.04-18, p<0.00001).

Conclusion: Combination analysis of HER2+ and LC, homo-HER2 has a higher rate in LCI type, hetero-HER2 has a higher rate in LCD. GC patients with hetero-HER2-LCD may not recommend trastuzumab as a first-line drug

Keywords: Gastric cancer; Immunohistochemistry; intratumoral HER2 heterogeneity; Lauren classification

Abbreviations

HER2: Human Epidermal Growth Factor Receptor 2; LC: Lauren Classification; GC: Gastric Cancer; OR: Odds Ratio; CI: Confidence Intervals; Hetero-HER2: Intratumoral Heterogeneously HER2 Positive; Homo-HER2: Intratumoral Homogeneously HER2 Positive; LCI: LC Intestinal Type; LCD: LC Diffuse Type; EGFR: Epidermal Growth Factor Receptor; IHC: Immunohistochemistry; FISH: Fluorescence *in situ* Hybridization

Introduction

According to the latest data from the World Health Organization, Gastric Cancer (GC) is the fifth most common cancer in the world, accounting for 6.8% of all cancers except melanoma skin cancer [1]. In the United States, GC accounts for 1.5% of all new cancers, with an estimated 26,240 new cases and an estimated 10,800 deaths in 2018 [2]. Despite declining morbidity and mortality, the costs and medical burden associated with GC have increased significantly [3,4]. GC is usually diagnosed as advanced and is defined as an unresectable local or metastatic disease with a poor prognosis and a five-year survival rate of no more than 5-20%.

Lauren-Classification (LC) and Human Epidermal Growth Factor Receptor 2 (HER2) status are two important pathological features of GC patients. LC is based on histomorphological structure and cell biological characteristics. The morphological structure and

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biological behavior, epidemiology, and molecular mechanisms of GC with different Lauren types are different. GC are classified into LC Intestinal (LCI), LC Diffuse (LCD), and mixed GC according to the LC criteria. The LC criteria are simple and clear, highly reproducible, and can better reflect the sex ratio and age distribution of GC patients. Although the application of LC system in GC was dated back to 1965, this classification is still extensively accepted and adopted by pathologists and physicians. Human Epidermal Growth Factor Receptor 2 (HER2), also known as c-erbB-2, neu, or p185, encodes a 185-kDa transmembrane glycoprotein located on the long arm of human chromosome 17 (17q12) [6]. It is expressed in many tissues and its main role in these tissues is to facilitate excessive/uncontrolled cell growth and tumorigenesis [7]. HER2 protein belongs to the epidermal growth factor receptor (EGFR) family. Dimerization of HER2 with activated EGFR molecules can activate downstream signaling pathways [8]. The possible effects of HER2 on tumors include controlling cell proliferation and migration [9]. Receptor dimerization results in the auto phosphorylation of tyrosine residues in the cytoplasmic domain of the receptors and triggers various signaling pathways leading to cell proliferation and tumorigenesis. HER2 overexpression is directly related to poor GC outcomes. In a study of 260 GCs, HER2 overexpression was an independent negative prognostic factor and its staining intensity was correlated with tumor size, serosal invasion, and lymph node metastases [10]. Other studies also confirmed the negative impact of HER2 overexpression in GC [11, 12], while others report that HER2 overexpression does not affect the prognosis of GC [13]. HER2 amplification is selective in GC. The HER2 positive rate of tubular adenocarcinoma is significantly higher than that of other types and its positive rate in moderately and highly differentiated GC is significantly higher than that in poorly differentiated GC [14]. Corresponding to the LC, the HER2 positive rate of LCI- GC is higher than the mixed and diffuse types.

The prognostic value of Lauren- HER2 status in GC remains unclear. The combination of LC and HER2 status may be more meaningful than use of any one factor. HER2-negative and LCI patients have the highest survival rates, while HER2-positive and LCD patients have the worst survival rates [15].

Kaito suggested intratumoral heterogeneously HER2 positive (hetero-HER2) in Immunohistochemistry (IHC) was an important predictor of clinical response and has a poor prognosticator for trastuzumab-based chemotherapy [16]. Thus, hetero-HER2 helps to further stratify patients with HER2-positive GC, and it should be considered in future clinical trials. In this study, we analyzed the relationship of LC, hetero-HER2 and intratumoral homogeneously HER2 positive (homo-HER2) in order to provide some clinical guide for GC pathological typing and treatment.

Materials and Methods

Search strategies

We searched PubMed, Embase and the Cochrane library as of October 2020 to identify the studies on HER2 heterogeneity and LC in GC patients. The language was limited to English and the search terms used were gastric cancer, stomach cancer, Lauren classification, human epidermal growth factor receptor 2, HER-2, HER2 and heterogeneity. Bibliographies of all relevant publications were scanned to identify relevant studies.

Inclusion and exclusion criteria

Studies meeting the following criteria were included: (1) the patients had surgical treatment and the disease was identified as GC pathologically; (2) assessment of intratumoral HER2 status were conducted by IHC, homo-HER2 was defined as every portion being HER2-positive by IHC, and if any portion of the tumor was HER2-negative, the tumor was defined as hetero-HER2; (3) the primary tumor was determined to be HER2-positive before trastuzumab treatment. Case reports, reviews, experimental studies, as well as studies that did not supply hetero-HER2 data were excluded. Figure 1 showed the flowchart of the included studies.

Data extraction and quality assessment

Quality of the enrolled studies was evaluated according to the Newcastle-Ottawa scale [17], which examines patient selection methods, comparability of study groups and assessment of outcomes. Studies that received 7-9 stars were considered high quality, 5-6 stars were of medium quality, and 5 or less stars were of low quality. Low-quality studies were excluded. We divided the patients into 4 groups according to their LC and HER2 status: heterog-HER2–Lauren intestinal type (hetero-HER2-LCI), homo-HER2–Lauren intestinal type (home-HER2-LCI), hetero-HER2–Lauren diffuse type (hetero-HER2-LCD) and homo-HER2–Lauren diffuse type (home-HER2-LCD).

Statistical analysis

Q statistic test [18] and the I-square test [19] were used to calculate the heterogeneity hypothesis and if significant heterogeneity ($P < 0.10$, $I^2 > 50\%$) was detected, a random effects model was used. Otherwise, we used a fixed effects model. Subgroup analyses of HER2-IHC (2+) or HER2-IHC (3+) were conducted. The summary Odds Ratio (OR) of home-HER2 vs. hetero-HER2 was the measure of interest. The overall significance was concluded by the OR and its 95% CI. RevMan (Version 5.4) [20] was used for the statistical analysis. The significance of the pooled OR was determined by Z test. $P < 0.05$ was considered statistically significant.

Results

197 studies were initially identified, of which six were eligible for final analysis. As shown in Table 1, 487 patients were included in the six studies. IHC was used to evaluate HER2 expression levels for all studies (Table 1).

Results of meta-analysis

487 samples with intratumoral HER2 positive were included in this study. The pooled data showed that for LC-intestinal type (LCI): hetero-HER2-LCI was observed in 212/301 (70%) and homo-HER2-LCD was observed in146/186 (79%) samples the hetero-HER2 was significantly lower than homo-HER2 in GC with LCI (OR=0.53, 95% CI: 0.23-1.47, p=0.01, Figure 2). For LC-diffuse type (LCD): hetero-HER2-LCD was observed in 89/301 (30%) samples and homo-HER2-LCD was detected in 38/186 (20%) samples, indicating that hetero-HER2-LCD was significantly higher than home-HER2-LCD in the GC with LCD (OR=1.94, 95% CI: 1.20-3.14. p=0.007, Figure 3).

Further staining intensity analysis of the HER2-IHC (2+) data, the results showed that hetero-HER2++ was observed in 97/301(32%) samples and homo-HER2++ in 14/186 (7%) samples, indicating that

Table 1: Major characteristics of the studies included in meta-analysis.

References	Year	Country	Patients number	Gender	IHC–HER2 antibody	Homo-HER2 patients				Hetero-HER2 patients			
				F/M		n	Age (yr.)	LCI	LCD	n	Age (yr.)	LCI	LCD
Kaito, et al. [16]	2019	Japan	88	27/51	4B5	65	69	48	15	23	67	14	9
Kurokawa, et al. [21]	2015	Japan	175	131/44	4B5	43	66	40	3	132	68	99	33
Lee, et al. [22]	2013	Korea	73	20/53	4B5	19	64	16	3	54	65	36	18
Motoshima, et al. [23]	2018	Japan	36	25-Nov	4B5	11	65	8	3	25	70	19	6
Wakatsuki, et al. [24]	2018	Japan	28	19-Jul	4B5	14	68	10	4	14	68.5	13	1
Yagi, et al. [25]	2019	Japan	87	30/57	4B5	34	62.5	24	10	53	68	31	22

F: Female; M: Male; IHC: Immunohistochemistry; HER2: Human Epidermal Growth Factor Receptor 2; LCI: Lauren Classification Intestinal Type; LCD: Lauren Classification Diffuse Type; Homo-HER2: Intratumoral Homogeneously HER2 Positive; Hetero-HER2: Intratumoral Heterogeneously HER2 Positive.

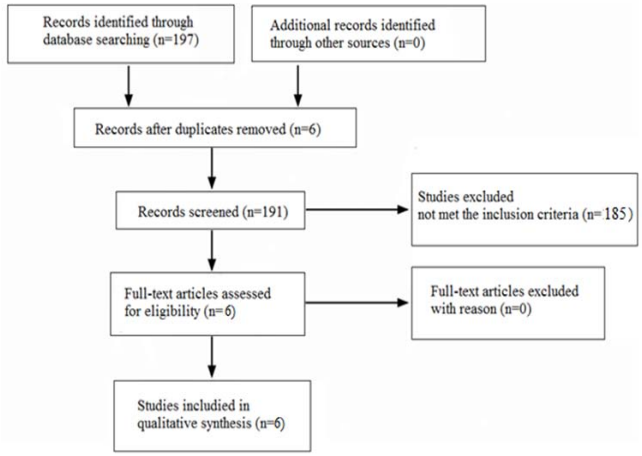

Figure 1: Flowchart of the literatures search.

hetero-HER2++ was significantly higher than home-HER2++ in the GC (OR=11.95, 95% CI: 5.57-25.64, p<0.00001, Fig. 4). HER2-IHC (3+) data revealed that hetero-HER2+++ was observed in 204/301(68%) samples and homo-HER2+++ was observed in172/186 (93%) samples, implying that hetero-HER2+++ was significantly lower than home-HER2+++ in the GC with IHC (3+) (OR=0.08, 95% CI: 0.04-18, p<0.00001, Figure 5).

Publication bias

The funnel plot was used for detecting publication bias. Little funnel plot asymmetry was found among the included studies, suggesting that the publication bias was statistically insignificant (Figure 6).

Discussion

The ToGA trial suggests that HER2 status (IHC2 + or IHC3 + / FISH +) is a predictor of response to trastuzumab therapy in patients with advanced GC and GJC [26, 27]. In recent years, the status of HER2 in GC patients has been extensively studied, and HER2 interpretation standards have been established and standardized [28]. HER2-positive GCs are mainly of the gut type [29-31]. GC heterogeneity is more common than in breast cancer, and it is estimated that heterogeneity can be as high as 79.3% in HER2-positive GC cases [32-34].

Diffuse GC is a type of Lauren type of GC. It has a low age of onset, low degree of cell differentiation, and diffuse growth. Mostly, it is poorly differentiated cancer, signet ring cell cancer, and undifferentiated cancer. The incidence is increased and the treatment effect is poor. Patients with intestinal GC have a better prognosis, and patients with intestinal GC have a higher incidence of HER2+++ positive.

Our analysis indicated that the dominant HER2 type for LCD is hetero-HER2 and homo-HER2 only account for 7% of tested samples, and which has a weak intensity of HER2-IHC staining [(IHC (2+)]. For LCI, the dominant HER2 type is home-HER2 [(IHC (3+)]. The LC should be referred for any samples with hetero-HER2 if HER2-IHC (3+). Aspects affecting trastuzumab efficacy in advanced GC are largely unidentified. Heterogeneity is a prominent feature of HER2 in GC patients. Many studies in recent years have shown that different LC types have significant differences in the efficacy of different chemotherapeutic drugs, and the same drugs have different effects on different classifications [26,35,36]. HER2 expression level is one of the factor affecting the efficacy. Although trastuzumab combined with chemotherapy extended the median overall survival

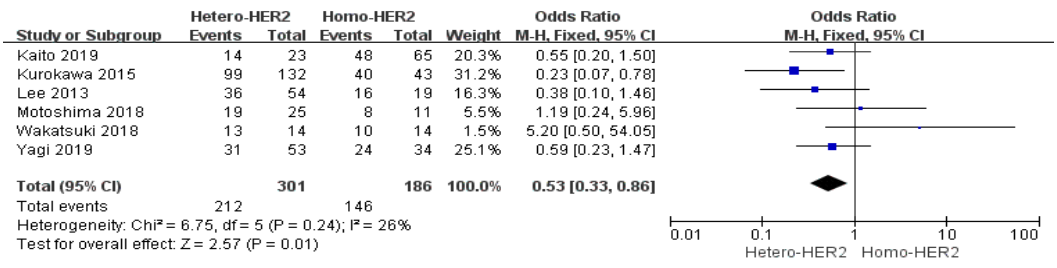

Figure 2: Forest plot showing the odds ratio of hetero-HER2 compared to homo-HER2 in patients with Lauren intestinal type.

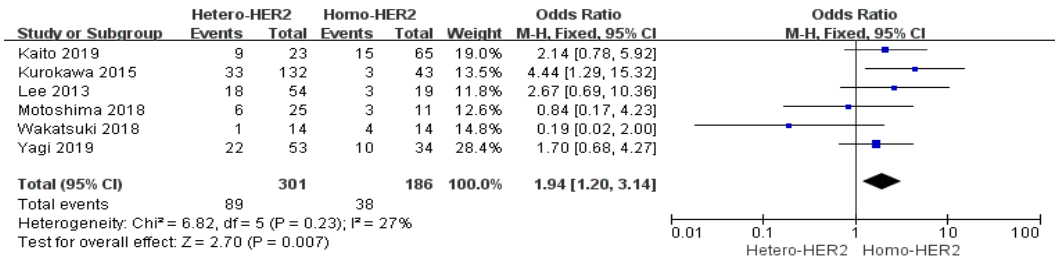

Figure 3: Forest plot showing the odds ratio of hetero-HER2 compared to homo-HER2 in patients with Lauren diffuse type.

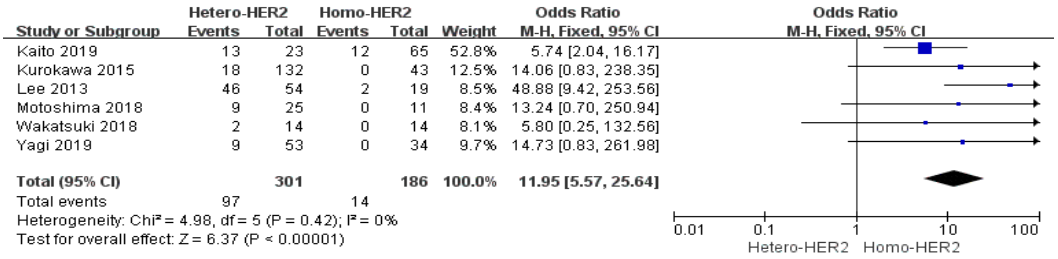

Figure 4: Forest plot showing the odds ratio of hetero-HER2 compared to homo-HER2 in patients with HER2-IHC (2+).

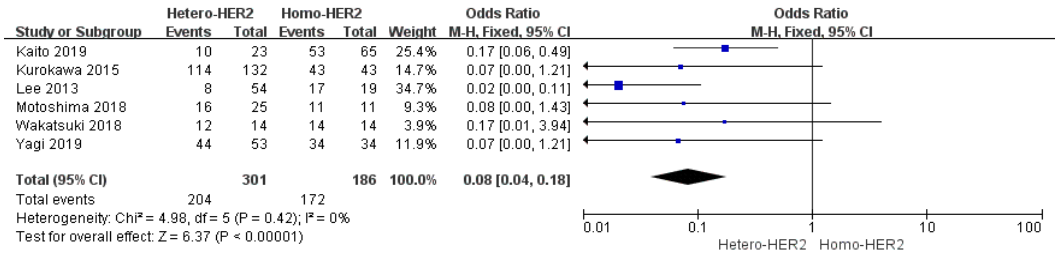

Figure 5: Forest plot showing the odds ratio of hetero-HER2 compared to homo-HER2 in patients with HER2-IHC (3+).

of patients with advanced GC to more than 1 year, this combination chemotherapy only benefits patients with LCI-GC, suggesting that hetero-HER2 is likely to be a subtype of GC, and it differ from home-HER2 and cannot be classified as HER2-positive GC for trastuzumab treatment [26]. Grabsch suggested GC with identical morphological phenotype have a different molecular phenotype and these are most likely requiring different treatment strategies [37].

HER2 expression persists as an important marker of trastuzumab for first-line systemic chemotherapy of GC. But compared with breast cancer, other strategies targeting HER2 did not improve prognosis in this GC molecular subtype. According to the HER2 biomarker testing guidelines, the apparent spatial hetero-HER2 overexpression has been considered as a major feature of the disease. A recent meta-analysis of eight studies [38] reported that HER2 had significant predictive ability for estimating OS with a hazard ratio (HR: 1.43; 95% CI: 1.09-1.88). Similarly, Wang et al. [39] that suggested that HER2 was poor prognostic feature with a HR of 1.59 (95% CI: 1.20-2.12). Liang et al. [40] suggested that HER2 overexpression was linked with Bormann type (I + II), well-differentiated intestinal type, lymph node metastasis, venous invasion and lymphovascular invasion. A recent multicenter study (consisting of 1148 GC patients undergoing gastrectomy from 11 institutes in Japan) found that HER2 overexpression to be an important predictive factor in patients with any stage of operable GC [21].

Hetero-HER2 is a true biological mark that is more frequent in gastric and GEJ cancers than breast cancer [41,42]. It is estimated that up to 30% of HER2-positive GCs have this heterogeneous feature [43]. Hetero-HER2 is much more significant than that in BC. In addition to tumor site, this heterogeneity of HER2 overexpression and amplification may be due to sample size, population diversity, interobserver variability, and inconsistent specimen processing and tumor sampling among different laboratories. When using FISH for HER2/NEU status assessment, the intratumoral heterogeneity could be more common because of the thinner field of view for diagnosis. Hence, it is difficult for a pathologist to report the exact status of HER2 overexpression and amplification [41]. Heterogeneity is more often found in IHC 2+ cases or mixed histological types [44] and HER2 overexpression is related to differentiated or intestinal types, lymphovascular invasion and lymph node metastasis [42,45,46].

LC and HER2 status are important for the GC outcomes. Prognosis for intestinal GC type is better [15]. However, the effectiveness of HER2 as an important clinicopathological factor is still controversial [47]. Studies of hetero-HER2 in breast cancer patients have produced inconsistent findings. Lee et al found that hetero-HER2 was a negative

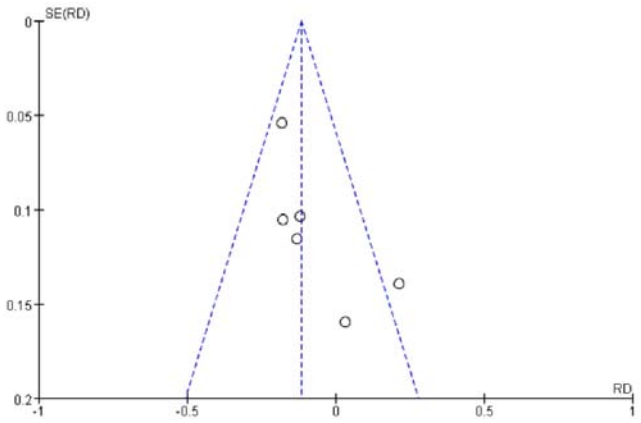

Figure 6: Funnel plot for publication bias of all studies included in this meta-analysis.

predictor of trastuzumab treatment [48,49]. However, the findings reported by Gullo et al. are the opposite [50].

Our analysis indicated that homo-HER2 had a higher rate in LCI, and 93 % of home-HER2-LCI samples were the HER2-IHC (3+), which should be given the trastuzumab. The patients with HER2-IHC (2+) had a much higher hetero-HER2-LCD rate. One study showed that hetero-HER2 was associated with a poor response rate to T-DM1 and pertuzumab compared to patients with no such heterogeneity in a phase II trial of HER2-positive breast cancer, meaning heterogeneity may need to be incorporated into treatment decisions in the future.

HER2 heterogeneous cancers may represent a distinct subset of HER2-positive GC, and these patients that may need different therapies. Therefore, we recommend that for all GC with HER2 positive, it should be further classified as hetero-HER2-LCI, homo-HER2-LCI, hetero-HER2-LCD and homo-HER2-LCD.

Given the high occurrence of intratumoral HER2 heterogeneity in GC, accurate HER2 assessment will need larger tissues and more detailed guidelines. The definition of hetero-HER2 should be re-assessed, particularly for HER2-IHC (2+) [51]. The mechanisms that cause hetero-HER2 are still largely unknown but may include neoplastic clones where HER2 is amplified/overexpressed in other HER2 negative tumor, or silence HER2 expression in a tumor region with homogeneous HER2 amplification. Six to eight biopsies are currently recommended and sufficient tissue for testing should be provided. Transcriptome analysis of RNA-seq data identified a signature that disclosed well-defined subtypes of diffuse-type GC: the intestinal-like (INT) and core diffuse type (COD) subtypes. This signature showed that in the other patient cohorts, the prognosis of LCD- GC is highly predictable and independent of clinical utility (HR 2.058, 95% CI 1.53–2.77, P = 1.76 × 10-6) and the LCD-GC can be further divided into molecular subtypes of INT and COD with different prognosis [52].

Strengths and Limitations

Regarding our meta-analysis, some restrictions should be mentioned. On literature searching, we tried our best to identify all relevant studies and retrieve additional unpublished information, but missing some data was unavoidable. Many factors such as age, gender, degree of differentiation, TNM staging, and postoperative treatment may be related to the predictive value of GC.

Conclusion

This meta-analysis shows that the home-HER2 with stronger staining intensity of IHC (3+) is the main HER2 type in LCI. The hetero-HER2 is the dominant HER2 type in LCD. The hetero-HER2 differs from home-HER2 and it cannot be classified as HER2-positive GC. In the case of IHC (3+), the hetero-HER2 sample should be referred to for Lauren classification and the GC patients with hetero-HER2-LCD may not recommend trastuzumab as a first-line drug.

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