

# HER2 protein expression correlates with Lauren classification and P53 in gastric cancer patients

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## Abstract

Human epidermal growth factor receptor 2 (HER2) is a key pathological characteristic of gastric cancer (GC). However, the clinical significance of HER2 expression in gastric carcinoma remains controversial. The purpose of this study was to analyze the clinicopathological characteristics of HER2 protein expression, Lauren classification and tumor protein p53 (P53) expression and to evaluate the clinical significance of HER2 protein expression. A total of 176 consecutive patients were prospectively recruited between January 2014 and December 2016 at the Second Affiliated Hospital of Zhejiang University School of Medicine. Histological analysis of the resected tissue was performed for HER2 protein expression using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Additionally, the expression status of HER2 protein and clinicopathological features were analyzed using the chi-squared ( $\chi^2$ ) test. Survival analysis was performed using the Kaplan–Meier method, and differences between the survival curves were determined using the log-rank test. All statistical analyses were conducted using SPSS 22.0 statistical software program (IBM Corp., Armonk, NY). A total of 176 patients with GC were enrolled in this study. Intratumoral heterogeneity of HER2 protein overexpression was observed in 42 of 176 cases with IHC grade 2+, accompanied by FISH positivity and IHC grade 3+. HER2 protein expression was correlated with tumor differentiation ( $P < .001$ ), Lauren classification ( $P = .001$ ), Borrmann type ( $P = .003$ ) and P53 expression ( $P < .001$ ). HER2 protein positivity was associated with significantly higher overall survival (OS) ( $P = .038$ ). Overexpression of HER2 protein was observed in 23.9% of the cases and was significantly related to the Lauren intestinal subtype and P53 negative expression. HER2 protein overexpression was independently associated with higher OS.

**Abbreviations:** FISH = fluorescence in situ hybridization, GC = gastric cancer, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry, Ki67 = marker of proliferation Ki-67, OS = overall survival, P53 = tumor protein p53, TNM = tumor-node-metastasis.

**Keywords:** fluorescence in situ hybridization, gastric cancer, human epidermal growth factor receptor 2, Lauren classification, P53

## 1. Introduction

Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide, after lung and liver cancer.<sup>[1]</sup> Although the incidence of GC has decreased worldwide, more than half of the cases occur in Eastern Asia, leading to significant public health problems in these countries, including China.<sup>[1,2]</sup> Despite advanced methods of early detection and multidisciplinary treatment of advanced GC, the 5-year survival remains unsatisfactory, although better results have been achieved in Japan and South Korea.<sup>[3]</sup>

Human epidermal growth factor receptor 2 (HER2) protein is a transmembrane receptor tyrosine kinase that plays an important role in the invasion and progression of GCs.<sup>[4,5]</sup> Overexpression of the HER2 protein was reported in 7% to

34% of GC patients and was considered an inversely prognostic factor.<sup>[6–8]</sup> It is important to note that the median overall survival (OS) time was longer in HER2 protein positive patients who received trastuzumab plus chemotherapy than in those who received chemotherapy only in phase 3 clinical trials (trastuzumab for GC or ToGA).<sup>[9]</sup> However, some studies have found no relationship between HER2 overexpression and survival.<sup>[10–12]</sup> In such studies, HER2 protein expression status was measured by IHC and the (normal, deletion, or amplification) status was evaluated by fluorescence in situ hybridization (FISH) analysis; patients with IHC 3+ or IHC grade 2+ and FISH+ were considered HER2 protein overexpression. Recently, several studies have reported clinical characteristics and prognosis related to HER2 protein overexpression in GCs.<sup>[12,13,14]</sup> However, the conclusions drawn from these studies

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All data generated or analyzed during this study are included in this published article [and its supplementary information files]

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varied. Therefore, the present study aimed to assess HER2 protein expression in a cohort of Chinese patients with GC, evaluate HER2 protein heterogeneity in GC, and determine the correlation of HER2 protein expression with clinicopathological features and OS in patients with GC.

## 2. Material and Methods

### 2.1. Patients

This retrospective study involved a cohort of 176 patients with GC who underwent curative surgery for primary GC at The Second Affiliated Hospital, College of Medicine, Zhejiang University, between January 2014 and December 2016. Patients enrolled in the study met the following criteria: patients were pathologically diagnosed with GC after gastrectomy by two experienced physicians, patients received no neoadjuvant chemotherapy or pre-radiotherapy, adequate paraffin-embedded tumor tissue sample for HER2 protein detection and clinicopathological analysis, no concurrent malignancy, and complete set of medical records for analysis were available. The Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medicine University, and the Second Affiliated Hospital, College of Medicine, Zhejiang University approved this study, and the patients provided written informed consent.

The following demographic and clinicopathological features were recorded and categorized: age (<60 and ≥ 60 years), sex, tumor differentiation, histological grade, Lauren classification (intestinal, diffuse, mixed), tumor protein p53 (P53), tumor location (proximal, distal), tumor size (≤5 and >5 cm), vascular invasion and nerve invasion, Borrmann type, and pathologic Tumor-Node-Metastasis (TNM) stage (according to the 8th American Joint Committee on Cancer TNM stage). It was clearly established that 56 patients had stage I disease, 44 had stage II disease, 61 had stage III disease, 15 had stage IV disease (Table 1). Clinicopathological data were obtained from the hospital information system (Electronic Medical Record System).

### 2.2. Immunohistochemistry

For all patients, the expression of HER2 protein, marker of proliferation Ki-67 (Ki67), and P53 were measured by two experienced pathologists using immunohistochemistry (IHC) and without advanced knowledge of clinical features. Histopathological parameters were measured using representative 4-μm thick paraffin-embedded tissue sections. IHC staining was performed using a primary polyclonal rabbit antibody (DAKO, Glostrup, Denmark) against HER2 protein on a Ventana Benchmark XT automatic staining system (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's instructions. To determine HER2 protein expression, the IHC scoring criteria proposed by Hoffmann et al were used as follows.<sup>[7]</sup> The sorting grades were as follows: grade 0, no membrane staining or membrane staining in <10% of the tumor cells; grade 1, faint or barely perceptible membranous reactivity in 10% or more of tumor cells; grade 2, weak to moderate reactivity in at least 10% of the cells, with staining across the lateral and basolateral membranes; grade 3, strong staining of the complete membrane in >10% of the tumor cells.<sup>[9]</sup> IHC scores of 0 and 1 + were regarded as negative for HER2 protein expression, while IHC 3 + was considered positive. For IHC 2 + patients, it is necessary to evaluate HER2 protein normal/deletion/amplification status by FISH analysis.<sup>[15]</sup> FISH analysis was performed using the PathVysion®HER2 DNA Probe kit (LSI®HER2/neu Spectrum Orange™/chromosome 7 CEP@17 Spectrum Green; Abbott Vysis, Downers Grove, IL) and the process was performed according to the kit manufacturer's instructions. HER2 protein was considered over-expressed when FISH showed HER2:centromere probe17 ≥ 2,

**Table 1**  
Correlations of HER2 protein expression status with clinical pathological characteristics of GC.

	HER2 status		P
	Positive (n = 42)	Negative (n = 134)	
Sex			.376
Male	33	96	
Female	9	38	
Age			.160
>60	31	83	
≤60	11	51	
Primary tumor			.655
T1	14	34	
T2	6	19	
T3	18	60	
T4	4	21	
Lymph nodes metastasis			.446
N0	22	52	
N1	6	20	
N2	5	21	
N3	9	41	
Distant metastasis			.950
M0	39	121	
M1	3	12	
Stage			.198
1	19	37	
2	9	35	
3	11	50	
4	3	12	
Tumor differentiation			<.001
Well	17	19	
Moderate	19	56	
Poor	6	59	
Tumor location			.857
Proximal	11	37	
Distal	31	97	
Borrmann			.003
1	7	6	
2	14	25	
3	21	97	
4	0	6	
Tumor size			.952
≥5	28	90	
<5	14	44	
Vascular invasion			.036
Positive	17	79	
Negative	25	55	
Nerve invasion			.075
Positive	12	59	
Negative	30	75	
Early stage			.186
Yes	14	31	
No	28	103	
Ki67			.095
Positive	38	106	
Negative	4	28	

GC = gastric cancer, HER2 = human epidermal growth factor receptor 2.

and the remaining were defined as negative. Ki67 and P53 were evaluated semi-quantitatively. Based on the level of nuclear staining, the scoring was initially classified using a scale from 0 to 4, as follows: 0, <10% staining; 1, 11% to 25% staining; 2, 26% to 50% staining; 3, 51% to 75% staining; 4, 76% to 100% staining. Finally, the cutoff value was established as previously described by Al-Moundhri et al.<sup>[16]</sup> When the staining of Ki67 was >25%, the sample was considered positive for the analysis. As for P53, a cutoff value of 10% was established. The similarity is that when the staining of P53 >10% and the staining of Ki67 was >25%, the sample displayed obvious nuclear staining.

### 2.3. Statistical analysis

Pearson chi-square ( $\chi^2$ ) test or Fisher exact test was used to compare differences among groups. Statistical significance was set at  $P < .05$ . Survival analysis was performed using the Kaplan–Meier method, and differences between the survival curves were determined using the log-rank test. All statistical analyses were conducted using SPSS 22.0 statistical software program (IBM Corp., Armonk, NY).

### 3. Results

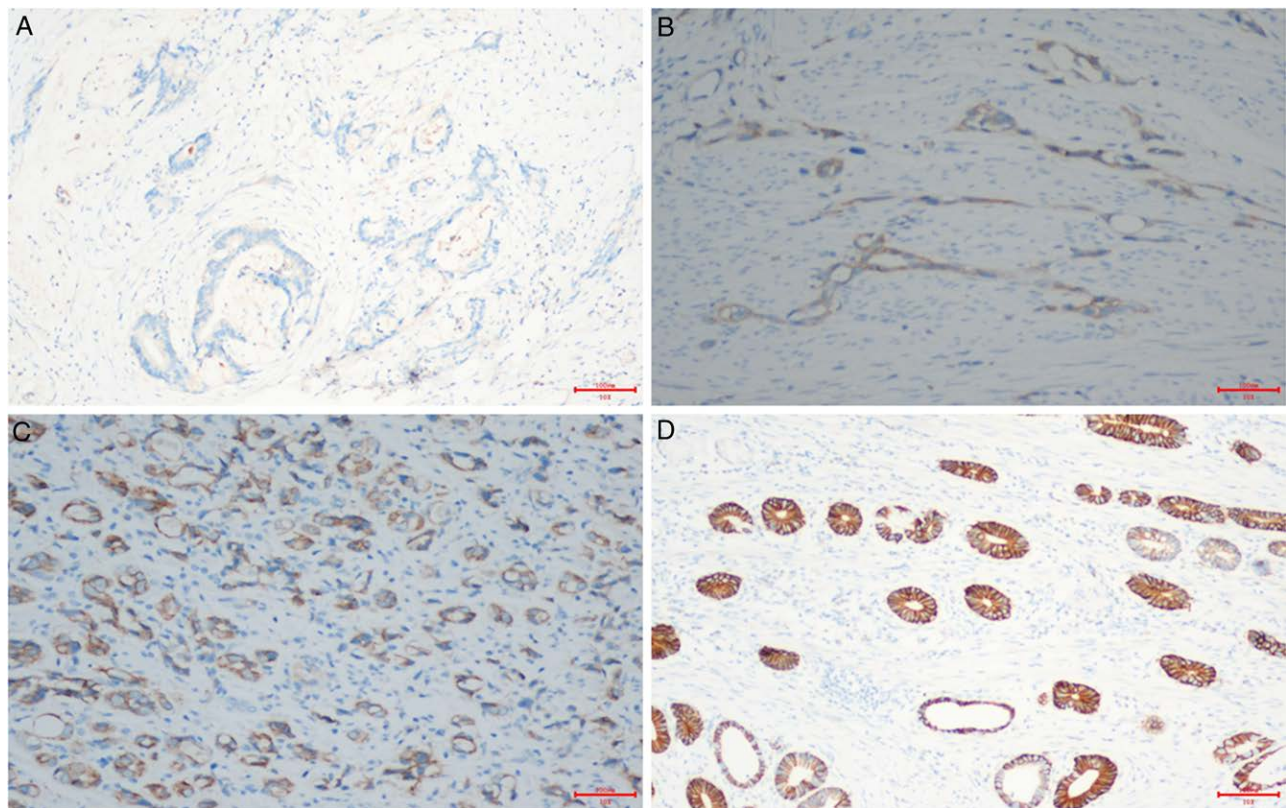
Among the 176 patients enrolled in this study, 129 were male and 47 were female, with a median age at presentation of 63.3 years (range, 34–87 years). All the patients underwent radical surgery for stomach carcinoma. Most tumors (72.7%,  $n = 128$ ) were located in the distal stomach, while 27.3% ( $n = 48$ ) were located in the proximal stomach. The majority (59.1%,  $n = 104$ ) were histologically classified as the intestinal subtype and Borrmann type III (67.0%,  $n = 118$ ) at presentation. Only 25.6% ( $n = 45$ ) had early-stage GC based on inspection of the surgical specimen tissues.

HER2 protein expression was measured using the methods mentioned above, and the results are shown in Figure 1. A total of 23.9% ( $n = 42$ ) of the patients were considered positive for HER2 protein expression, whereas 76.1% ( $n = 134$ ) of the patients were considered negative (Table 2). The results of univariate analysis comparing the clinicopathological features of GC patients with HER2 protein status are presented in Table 1. HER2 protein-positive GCs were prominent among patients older than 60 years of age (27.2%, 31/114) and were also predominant in men (25.6%, 33/129). In addition, HER2 protein positivity was more commonly detected in distal tumors

(24.2%, 31/128), although the difference was not statistically significant ( $P = .857$ ).

Most patients 72.7% ( $n = 128$ ) presented with advanced (T2 and above) tumors and 21.9% ( $n = 28$ ) were HER2 protein-positive. Additionally, among the 45 patients with early-stage GC, 31.1% ( $n = 14$ ) were HER2 protein positive, but this was not statistically significant ( $P = .186$ ). Among the 74 patients without lymph node metastasis, 29.7% ( $n = 22$ ) were HER2 protein positive. Among the 102 patients whose resected gastrectomy specimens showed lymph node metastasis, 19.6% ( $n = 20$ ) were HER2 protein-positive. As for M (metastasis) stage patients, cross-sectional image-based staging revealed that 8.52% ( $n = 15$ ) of patients were in the M1 stage (distant metastases present), whereas 20.00% ( $n = 3$ ) of these patients were HER2 protein positive (Table 1). The expression of P53 IHC was shown in Figure 2. In our study, 72.2% ( $n = 127$ ) of the patients were P53 positive and the patients with positive P53 expression had a higher proportion of negative HER2 protein expression ( $P < .001$ ). Similarly, most tumors (59.1%,  $n = 104$ ) belonged to the intestinal subtype, and the intestinal subtype expressed higher levels of HER2 protein (33.7%,  $n = 35$ ). A significant correlation was observed between HER2 protein overexpression and the histological subtype of GC ( $P = .001$ ) (Table 3).

According to the univariate analysis, there was a significant association ( $P < .001$ ) between HER2 protein positivity and high histological differentiation of the tumor. Specifically, 20.5% ( $n = 36$ ) of the patients had histologically highly differentiated tumors, while 47.2% ( $n = 17$ ) of the patients overexpressed HER2 protein, indicating that the overexpression of HER2 protein is more common in GC patients with high-grade differentiated tumors (Table 1). As shown in Table 1, HER2 protein positivity was associated with Borrmann classification



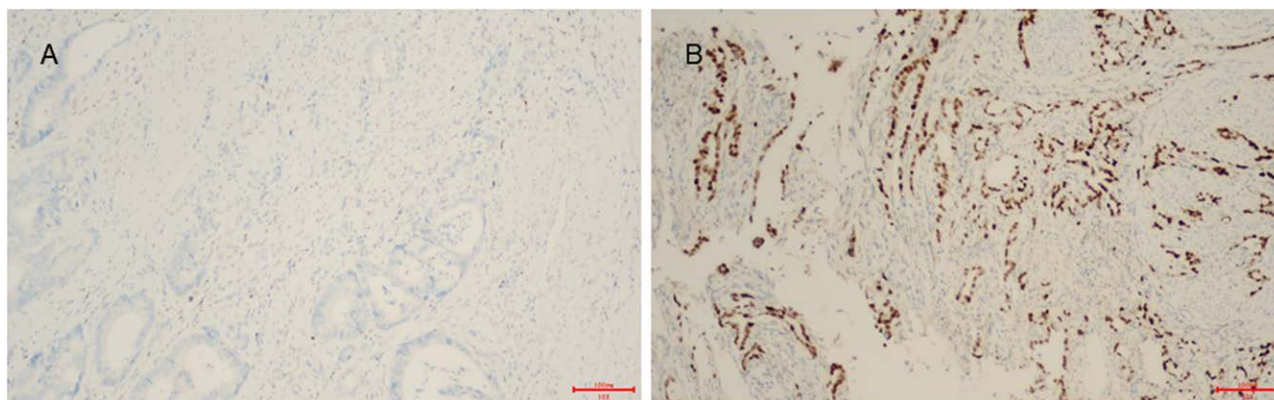
**Figure 1.** Expression of HER2 protein in the tested samples detected by immunohistochemistry (x10). (A) Immunostaining shows no staining on tumor cell membrane. (B) Immunostaining shows positive reaction (1+). (C) Immunostaining shows positive reaction (2+). (D) Immunostaining shows positive reaction (3+) with complete or basolateral membranous staining. HER2 = human epidermal growth factor receptor 2.



**Table 2**  
IHC combined with FISH defining HER2 protein expression status.

	HER2 negative			HER2 positive	
	0	1+	2+/FISH(-)	2+/FISH(+)	3+
No. (%)	74 (42.0)	38 (21.6)	22 (12.5)	6 (3.4)	36 (20.5)
Sum. (%)	134 (76.1)			42 (23.9)	

FISH = fluorescence in situ hybridization, HER2 = human epidermal growth factor receptor 2, IHC = immunohistochemistry.



**Figure 2.** The expression of P53 immunohistochemistry (×10). (A) Negative expression of P53. (B) Positive expression of P53. P53 = tumor protein p53.

**Table 3**  
Correlations of HER2 protein expression status with Lauren classification and P53 of GC.

	Lauren classification			P	P53		P
	Intestinal (n = 104)	Diffuse (n = 38)	Mixed (n = 34)		Positive (n = 127)	Negative (n = 49)	
HER2 status				<b>.001</b>			<b>&lt;.001</b>
Positive (n = 42)	35	3	4		21	21	
Negative (n = 134)	69	35	30		106	28	

GC = gastric cancer, HER2 = human epidermal growth factor receptor 2, P53 = tumor protein p53.

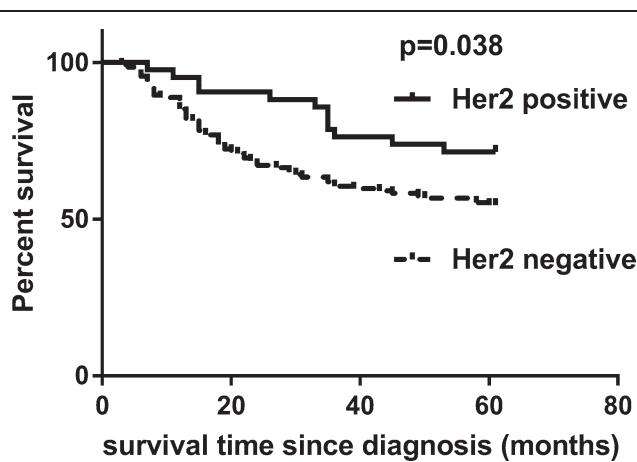
( $P = .003$ ) and vascular invasion ( $P = .036$ ). However, there was no statistically significant association between HER2 protein positivity and Ki67 expression ( $P = .095$ ), nerve invasion ( $P = .075$ ), tumor size ( $P = .952$ ), or early tumor stage ( $P = .186$ ).

All patients were followed up for 5 years, or death as the endpoint. We have excluded these patients who were lost to follow-up when we enrolled in this study. In our current study, OS was better among HER2 protein positive patients, and the differences in survival curves (Fig. 3) were statistically significant ( $P = .038$ ).

#### 4. Discussion

Despite a worldwide decline in its incidence, GC remains one of the most common malignancies in China, where its incidence (29.9 new diagnoses per 100,000 people) remains high.<sup>[17]</sup> According to epidemiological research worldwide, GC predominantly affects elderly males.<sup>[17,18]</sup> In the present study, 64.8% (114/176) of the patients were 60 years old or older. The Lauren classification, tumor location, HER2 protein expression status, tumor differentiation, and tumor size are essential clinicopathological features reported in GC research. In our study, HER2 protein was an important clinicopathological characteristic that we investigated to determine the association between the expression of HER2 protein and other GC-related factors.

In the current study, we found an overall HER2 protein positivity rate of 23.9% in patients with GC. This finding is similar to those of various other studies from American and European researchers who have reported GC HER2 protein positivity rates ranging from 10% to 22.8%.<sup>[4,5]</sup> However, some Asian studies have reported rates ranging from 11.7% to 15.74%.<sup>[14,19,20]</sup> The importance of the HER2 protein status in GC remains controversial, and an increasing number of researchers are studying the significance of HER2 protein expression in GC. In general, the Lauren classification can be divided into intestinal, diffuse, and mixed subtypes. In this study, a significant relationship was observed between HER2 overexpression and the intestinal subtype of GC. Specifically, intestinal subtype patients were more likely to be HER2 protein-positive than the other two subtypes. Our results are also similar to those of most reported studies, which revealed that the intestinal histological subtype was predominant in HER2 protein-positive patients.<sup>[12,13,15,21]</sup> In addition, HER2 protein overexpression was associated with differentiated histology. High rates of HER2 protein-positive GCs were detected among well-differentiated and moderately differentiated GCs compared to poorly differentiated GCs. This result is similar to that reported by Oh et al, which indicated that HER2 protein overexpression was associated with the intestinal subtype and well moderately differentiated GC tumors.<sup>[22]</sup> In addition, the positive rate of HER2 protein expression was 31.3% higher in GC patients with negative vascular invasion



**Figure 3.** Kaplan–Meier curves for 176 gastric cancer patients according to HER2 protein expression status. HER2 = human epidermal growth factor receptor 2.

(25/80), and the result was statistically significant ( $P = .036$ ). Some studies have reported findings similar to those of the present study regarding the anatomical location of the tumor, indicating that there is no difference between distal and proximal GC tumors.<sup>[2,11,23]</sup> The efficacy of trastuzumab in breast cancer therapy has led to an emerging interest in its therapeutic effects in patients with HER2 protein positive GC. The ToGA trial, a randomized controlled multicenter phase III study, was designed to evaluate the efficacy of trastuzumab (an anti-HER2 protein drug) in combination with chemotherapy for the treatment of advanced gastric carcinoma.<sup>[9]</sup> The overexpression rate of the HER2 protein in the ToGA study was 22.1%, which is similar to our findings. Additionally, as in our study, HER2 protein overexpression was advantageous in the intestinal GC type compared to the other two types. However, the ToGA study revealed that HER2 protein overexpression correlated with the location of the tumor, indicating that the tumor was prevalent in the proximal stomach. The lack of a significant correlation between HER2 protein overexpression and tumor location in our study could be explained by the small number of enrolled patients.

In China, a study conducted by Qiu et al in 2014 analyzed the immunohistochemical expression in 838 GC cases and found prevalent HER2 protein overexpression in 11.2%.<sup>[15]</sup> They found predominant HER2 protein expression in the intestinal GC type and detected a significant association between the overexpression of HER2 protein and proximal GC. The frequency of HER2 overexpression was much lower than that observed in the present study. The apparent discrepancies between various other studies and our study can be reconciled if we consider the immunohistochemical method used and the differences among the enrolled patients. A second study conducted at Sun Yat-sen University by Liu et al in 2016 found HER2 protein overexpression in 40.3% of 678 patients analyzed.<sup>[24]</sup> Such a high rate of HER2 protein overexpression may be due to the enrolled patients, which only included stage I-III GC patients, whereas our study included stage I-IV GC patients according to the eighth edition of the American Joint Committee on Cancer TNM staging system. In addition, both studies concluded that HER2 protein overexpression was significantly correlated with tumor histological classification, and HER2 protein was preferentially expressed in the intestinal subtype of GC tumors. Moreover, the GC intestinal subtype was observed more frequently in well-differentiated and lower-stage tumors. Accordingly, it is not difficult to explain why the study by Liu et al found higher HER2 protein overexpression than our current study. A review of previous research revealed that the Lauren classification was considered one of the most important predictive factors of prognosis in GC.<sup>[25]</sup> In addition, the intestinal

subtype and well-differentiated tumor cells have a consequential relationship with better prognosis.<sup>[25,26]</sup>

HER2 protein overexpression was regarded as the most crucial advancement in gastric carcinoma in the coming years at the American Society of Clinical Oncology annual meeting in 2014. In the current study, we compared our results with the clinicopathological characteristics of GC patients. The expression rate of P53 in IHC analysis was 72.2% (127/176). Additionally, HER2 protein expression significantly correlated with P53 expression ( $P < .001$ ). In addition, according to previous studies, there is a correlation between HER2 protein expression status and P53 nuclear staining, but further research on this relationship is warranted.<sup>[27]</sup> Moreover, P53 expression was significantly correlated with some clinicopathological features: HER2 protein expression ( $P = .04$ ), age below 60 years ( $P = .03$ ), tumors >5 cm in size ( $P = .01$ ), and Ki67 ( $P = .0001$ ).<sup>[16]</sup> Furthermore, in the current study, overexpression of P53 was considered an independent prognostic factor.<sup>[28]</sup> Younger patients with larger tumors and a high proliferation index, as measured by the level of Ki67 expression, indicate more aggressive biological behavior of tumor cells and have worse prognosis.<sup>[29,30]</sup> However, Moundhri et al reported no relationship between P53 and sex, tumor location, Lauren classification, T-stage, and lymph node metastasis.<sup>[16]</sup> Similarly, we found that there was a significant difference between HER2 protein overexpression and P53 expression ( $P < .001$ ). However, in the current study, we did not find a significant correlation between P53 and age, sex, tumor size, Ki67 and some additional clinicopathological factors. As mentioned above, overexpression of HER2 protein was not the same as the prognosis of GC patients. Our study showed that HER2 overexpression was associated with increased OS ( $P = .038$ ). In our study, the vast majority of the patients with HER2 protein overexpression were treated with trastuzumab-targeted therapy, except for a small number of patients who did not use Herceptin for economic reasons. Better histological type and subsequent targeted therapy may be associated with a better prognosis in HER2 positive patients.

Our study had several inherent limitations. First, this was a retrospective study rather than a prospectively designed study, and the data were limited. Our research was a single-center investigation, and selection bias was inevitable, while the benefit of a single center was that we could ensure the accuracy and consistency of the research methods and data. Selection bias at patient enrollment may have implications for prognostic and correlation analyses. The potential mechanism of HER2 protein overexpression as well as that of P53 overexpression in intestinal type GC require further experimental study.

### 5. Conclusions

Our IHC-based study found a HER2 protein overexpression rate of 23.9% in patients with GC. HER2 protein overexpression is more likely to occur in Lauren intestinal subtype and P53 negative GC patients. HER2 protein overexpression was independently associated with higher OS.

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### Author contributions

Yiming Chu is the first author of this manuscript. Yiming Chu designed, acquired and analyzed the data, drafted and revised the manuscript. The data was also acquired and analyzed by

Hongbo Li. The conception, design, and final approval of the submitted version were performed by Qingqu Guo. Conception and design were also done by Dan Wu.

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