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Prognostic Factors and Clinical Characteristics in Patients with Resectable Human Epidermal Growth Factor Receptor-2 Positive Gastric Cancer

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Abstract

Background: Gastric cancer is the 4th most common cancers worldwide. The discovery of Human Epidermal Growth Factor Receptor 2 (HER2) has altered the treatment paradigm in advanced gastric cancer. However, the prognostic role of HER2 in resectable gastric cancer remained undetermined.

Methods: We retrospectively analyzed 32 patients with HER2-positive and 232 patients with HER2-negative resectable gastric cancer from Jan 2012 to Dec 2018 in a medical institute in Taiwan. Clinical characteristics, histology features, selected treatments and survival outcomes were described. Prognostic factors for Recurrence-Free (RFS) and Overall Survival (OS) were evaluated using uni- and multivariate regression by Cox proportional hazard model.

Results: HER2 positivity was 12.1% in total included patients. The age, sex, disease stage, lymph node metastasis, performance status, Helicobacter pylori colonization and primary tumor location were well-balanced. HER2-positive patients were associated with intestinal type tumor to the contrary of diffuse type in negative ones, similar in mixed type and higher median serum cancer antigen-199 (intestinal/diffuse type in HER2-(+) *vs.* (-), 82.1/15.6% *vs.* 41.1/38.3%, p<0.05; mixed type, 6.3% *vs.* 17.2%, p=0.128) (CA199 in HER2-(+) *vs.* (-), 16.49 U/mL *vs.* 14.10 U/mL, p=0.03). Most patients received operation with/without adjuvant chemotherapies (HER2-(+) *vs.* (-), 87.6% *vs.* 91.8%). Only few HER2-positive patients received HER2-directed therapies upon frontline or recurrent condition. Patients with advanced stage, extensive lymph node metastasis, tumor vascular invasion and higher CA199 had pronounced poor RFS and OS. HER2 positivity correlated with a shorter but not significant RFS (HER2-(+) *vs.* (-), median RFS 18.2 *vs.* 54.0 months, HR=0.84, p=0.51) and a significantly shorter OS (HER2-(+) *vs.* (-), median OS 30.0 *vs.* 79.2 months, HR=0.61, p=0.04). Uni- and multivariate regressions indicated that poor performance status, lymph node metastasis, high CA199, primary tumor location and tumor vascular invasion predicted adverse survival outcome.

Conclusion: Patients with resectable HER2-positive gastric cancer are associated with intestinal type tumor and are undertreated with HER2-directed therapies HER2 status alone is not a prognostic factor for RFS but predicts poor OS. Performance status, lymph node metastasis, high CA199, primary tumor location and tumor vascular invasion predicted the survival outcome.

Keywords: HER2; Gastric Cancer; Trastuzumab; High CA199

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Background

Gastric cancer is one of the prevalent upper gastrointestinal malignancies and ranks the 4th in the most common cancers worldwide [1]. The burden of gastric cancer had persistently increased in the developing world, notably in Asia, Latin America and Eastern Europe, where nearly half of the new cases diagnosed in advanced diseases [2]. Although the outcome of localized resectable diseases had dramatically improved recently, almost two third of high-risk patients experience recurrence

and progression. In addition to curative gastrectomy, peri-operative chemotherapy had been implicated to strengthen the surgical outcome in patients with resectable gastric cancer [3-5]. However, the general result of recurrent or advanced diseases remains unsatisfactory, with a median survival of 4 to 12 months given aggressive salvage therapies [6].

The discovery of Human Epidermal Growth Factor Receptor 2 (HER2/neu, or ERBB2) sheds new lights as a promising therapeutic target in gastric cancer. HER2 serves as a transmembrane tyrosine kinase receptor that regulates proliferation, differentiation and tumor aggressiveness [7]. Identified either by target gene amplification, protein overexpression or both, HER2-positive diseases account for 5% to 20% in total gastric or gastroesophageal adenocarcinoma and range broadly according to ethnicity and geographic distribution [8-12]. HER2-directed agents such as trastuzumab, a monoclonal antibody against HER2 dimerization, had provided therapeutic superiority in combination with chemotherapy in advanced diseases [13]. However, the role of HER2-directed therapies in resectable early or locally advanced gastric cancer remained vastly undetermined.

HER2 positivity not only serves as a therapeutic predictor but also a possible prognostic factor. Confounded by inconsistent HER2 definition and patient population, most studies suggested that HER2 positivity correlated with pronounced recurrence and worse survival yet some indicated a similar outcome to HER2-negative patients [14,15]. In addition, the correlation of HER2 positivity to recurrence and surgical outcome in resectable diseases remained to be delineated as prognostic factors in early gastric cancer. Together these results may consolidate that patients with early HER2-positive gastric cancer are potential candidates for HER2-directed adjuvant therapies.

In the present study, we retrospectively analyzed 32 patients with HER2-positive disease out of 264 patients with resectable gastric cancer in a tertiary medical institute. Clinical characteristics, histology features, biochemical markers and selected treatments were described. The prognostic factors, such as HER2 positivity, were analyzed to unveil the interactions with recurrence or death in early gastric cancer. An overview of the recent discoveries, ongoing trials and the correlation with molecular profiling concerning Resectable HER2-positive gastric cancer is reviewed as well.

Materials and Methods

Patients

A total of 32 Han Chinese patients with Resectable HER2-positive gastric cancer from Jan 2012 to Dec 2018 were included. The resectability of the disease was referred to an initial clinical staging of I to III or a re-staging of I to III after neoadjuvant therapies in the absence of metastatic tumors. Another 232 patients with HER2-negative disease were included in the identical time period and institution for comparisons. All included patients had received diagnosis and treatment in National Cheng Kung University Hospital, a tertiary medical institute in Tainan, Taiwan. Patients were either diagnosed through surgical or biopsied specimens but not cytological specimens from body fluid or tissue aspirations. Essential criteria for inclusion were patients with confirmed gastric (cardia, antrum, fundus, body and pylorus) or Gastroesophageal Junction (GEJ) (tumor center 2 cm to 5 cm below GEJ occupying lower esophagus or proximal gastric cardia) carcinoma. HER2 positivity was defined by Immunohistochemistry (IHC) of a 4-titered scoring system and prepared with human primary monoclonal HER2 antibodies (Biogenex CB11 or Ventana 4B5). A cut-off value of a 10% stained tumor cells is recognized positive, according to the College of American Pathologist (CAP) approved guideline [16,17]. Fluorescence in Situ Hybridization (FISH) of target HER2 amplification was utilized in addition to IHC results. HER2 gene copy number per tumor cell were calculated and compared with chromosome *via* Chromosome Enumeration Probe 17 (CEP17) in at least 20 dividing cells, according to manufacturers' instructions [17]. FISH positivity was defined as HER2/CEP17 ≥ 2.0. "HER2-positive" disease was then determined by an IHC of (3+) or (2+) plus FISH-positive, and "HER2-negative" as IHC 0, (1+) or (2+) plus FISH-negative. Of the tumors with multiple samplings and heterogeneous results, positive disease was defined at least one sampling positive for HER2. Patients with incomplete HER2 status determination, multiple primary cancers or cancer of an unknown primary site were excluded from the study.

Clinical evaluations

Included patients had received at least one of the primary treatments, including gastrectomy (subtotal, partial or total) plus perigastric/celiac axial lymph node Dissection (D1/D2) with/without adjuvant chemotherapy, systemic chemotherapy, radiotherapy, Concurrent Chemoradiotherapy (CCRT), HER2-directed therapy or supportive care. Subsequent treatments referred as any salvage or palliative treatments after recurrence, progression or failure of the primary one. Clinical and pathological characteristics, including demographic features, biomarkers, presence of *Helicobacter pylori* (*H. pylori*), and treatment response and survival time intervals were evaluated in the medical records. Overall Survival (OS) was defined by the date of diagnosis to death of the patient at any causes. Recurrence-Free Survival (RFS) was defined by the date of diagnosis to first disease progression, recurrence, clinician-judged subsequent treatment initiation, or death of the patient at any causes.

Statistical analysis

Clinical characteristics were presented with descriptive analyses in case numbers or percentages. Categorical variables were compared by chi-square test with Yates correction or Fisher's exact test. Continuous variables were compared by independent Student's t-test or Mann-Whitney U test. OS and RFS were evaluated by Kaplan-Meier survival estimation and compared with log-rank test. Relevant clinical variables were evaluated as prognostic factors for OS or RFS by Cox proportional hazard regression model. We performed univariate regression and selected the covariates if a significant $p<0.05$ or clinically-relevant variables into multivariate regression. All independent covariates were tested for multiple collinearity and excluded if so. The fitness of Cox proportional model was also confirmed without violations. All statistical significance was indicated if $p<0.05$ or else specified. GraphPad Prism 7.0® (GraphPad software, CA, US), R 3.5.1® (R statistics, Vienna, AT) and IBM SPSS STATISTICS 22.0® (SPSS Inc., IL, US) were used for data management and computing.

Results

Patient characteristics

Clinical and tumor characteristics are presented in Table 1. Of the included patients, HER2 positivity was 12.1%. The median age (HER2-(+) *vs.* (-), 66.0 *vs.* 67.1 years) and sex distribution (male in HER2-(+) *vs.* (-), 65.6% *vs.* 53.0%) were similar with a slight male predominance. All HER2-positive patients were HER2-IHC

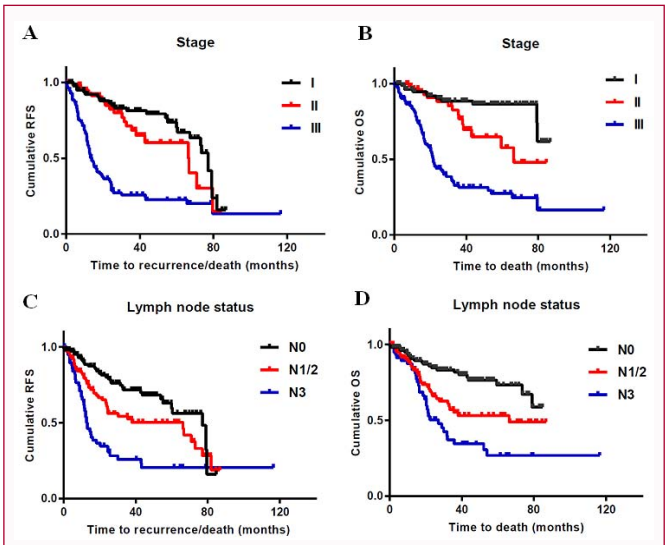

Figure 1: Kaplan-Meier survival estimation.
RFS and OS by stage (A-B) and lymph node metastasis (C-D)
(A) RFS: Stage I (n=88) *vs.* stage II (n=61), p=0.17; stage II (n=61) *vs.* stage III (n=115), p<0.001*; stage I (n=88) *vs.* stage III (n=115), p<0.001*
(B) OS: Stage I (n=88) *vs.* stage II (n=61), p=0.02*; stage II (n=61) *vs.* stage III (n=115), p<0.001*; stage I (n=88) *vs.* stage III (n=115), p<0.001*
(C) RFS: N0 (n=108) *vs.* N1/2 (n=98), p=0.02*; N1/2 (n=98) *vs.* N3 (n=58), p=0.01*; N0 (n=108) *vs.* N3 (n=58), p<0.001*
(D) OS: N0 (n=108) *vs.* N1/2 (n=98), p=0.004*; N1/2 (n=98) *vs.* N3 (n=58), p=0.03*; N0 (n=108) *vs.* N3 (n=58), p<0.001*
Log-rank test, p<0.05 as statistically significant and shown in*

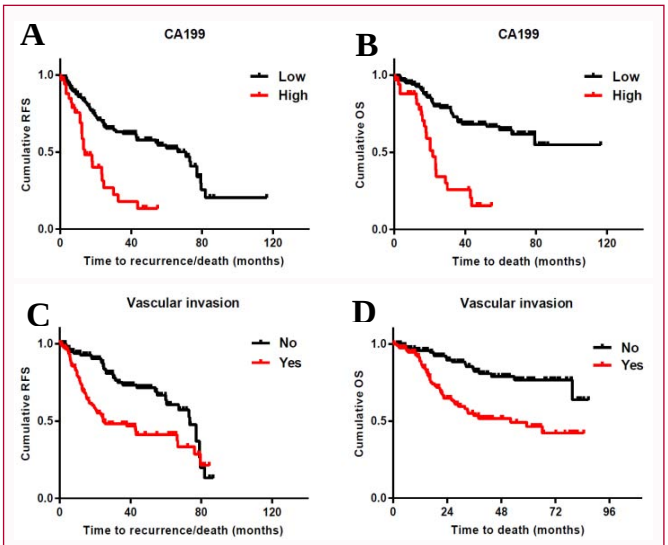

Figure 2: Kaplan-Meier survival estimation
RFS and OS by CA199 (A-B) and tumor vascular invasion (C-D)
(A) RFS: low (n=230) *vs* high (n=34), p=0.001*
(B) OS: low (n=230) *vs* high (n=34), p<0.001*
(C) RFS: no (n=144) *vs* yes (n=120), p=0.01*
(D) OS: no (n=144) *vs* yes (n=120), p<0.001*
Log-rank test, p< 0.05 as statistically significant and shown in*

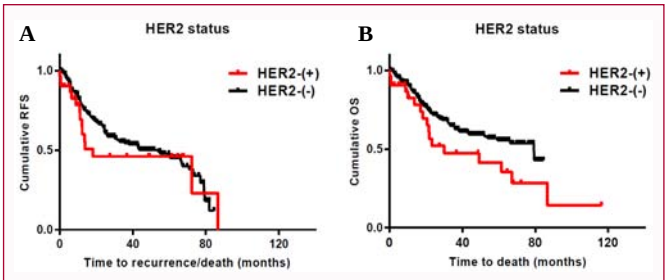

Figure 3: Kaplan-Meier survival estimation.
RFS and OS by HER2 status (A-B)
(A) RFS: negative (n=232) *vs.* positive (n=32), p=0.51
(B) OS: negative (n=232) *vs.* positive (n=32), p=0.04*
Log-rank test, p<0.05 as statistically significant and shown in*

(3+) or (2+) plus HER2-FISH (+) and most of the HER2-negative patients were IHC 0 (85.8%). The majority of the patients were fair in performance status at diagnosis (Eastern Cooperative Oncology Group (ECOG) 0 or 1 in HER2-(+) *vs.* (-), 87.5% *vs.* 87.5%), had an early disease (stage I to IIIA in HER2-(+) *vs.* (-), 81.3% *vs.* 78.4%), had the primary tumor at stomach (stomach in HER2-(+) *vs.* (-), 96.9% *vs.* 91.8%). Regardless of the HER2 status, the disease stage, lymph node metastasis, performance status and primary tumor location were well-balanced between HER2-positive and -negative group. We found 1/4 of the patient's positive for *H. pylori* colonization at diagnosis and most were detected *via* microscopic visualization or rapid antigen test. Lauren histology classification was significantly distinctive between the two groups, with a pronounced intestinal type in HER2-positive patients and diffuse type with signet ring cells in HER2-negative patients (intestinal/diffuse type in HER2-(+) *vs.* (-), 82.1/15.6% *vs.* 41.1/38.3%, p<0.05) and similar in the mixed type (mixed type in HER2-(+) *vs.* (-), 6.3% *vs.* 17.2%, p=0.128). Median serum Carcinoembryonic Antigen (CEA) was slightly elevated in HER2-positive patients but not statistically significant (CEA in HER2-(+) *vs.* (-), 2.92 ng/mL *vs.* 2.10 ng/mL, p=0.08). However, median serum cancer antigen-199 (CA199) was significantly higher in HER2-positive patients (CA199 in HER2-(+) *vs.* (-), 16.49 U/mL *vs.* 14.10 U/mL, p=0.03). Few patients (3/264) had received preoperative therapies and 45.8% of patients had a disease recurrence or progression in our cohort. We found only 3/32 HER2-positive patients ever received HER2-directed therapy upon recurrence or progression but none in the frontline or postoperative adjuvant condition.

Selected treatments

Curative operation remained the major primary treatment regardless of the HER2 status in our cohort (HER2-(+) *vs.* (-), 87.6% *vs.* 91.8%) (Table 2). Adjuvant chemotherapies were conducted in 1/3 of patients according to physician's preferences. In the total of 10 patients receiving adjuvant chemotherapies with locally advanced HER2-positive disease, 5 had 5-Fluoropyrimidine (5-FU)/platinum-doublet, 3 had capecitabine/oxaliplatin (XELOX), 1 had gimeracil/oteracil/tegafur (S-1) and 1 had oral 5-FU. In the HER2-negative patients receiving, 37% of patients had S-1, 32% had 5-FU/platinum-doublet, 18% had XELOX and 13% had 5-FU-based adjuvant chemotherapies. None of patients with HER2-positive disease had received frontline HER2-directed therapies in our cohort. In the subsequent treatments after recurrence or progression of the disease, more patients received systemic therapies (HER2-(+) *vs.* (-), 72.7% *vs.* 56.2%) (Supplementary 1). Only 3/11 patients with HER2-positive disease had HER2-directed therapies (XELOX plus trastuzumab, n=2; lapatinib, n=1). In the second-line systemic treatments, 2 had capecitabine, 1 had XELOX and 1 had epirubicin/cisplatin/5-FU (ECF) in the total of 4 patients with HER2-positive disease; 11 had XELOX, 9 had taxane-based, 6 had 5-FU/oxaliplatin/folic acid (FOLFOX), 5 had pembrolizumab, 5 had oral 5-FU, 4 had platinum-based chemotherapies and 1 had S-1 in the total of 41 patients with HER2-negative disease. Few patients received Concurrent

Table 1: Patient and tumor characteristics.

	All patients (n=264)	HER2-positive (n=32)	HER2-negative (n=232)	p
Age, years				
Median (IQR)	67.1 (58.6-76.9)	66.0 (62.8-77.2)	67.1 (57.8-76.9)	0.813
Sex, n (%)				
Male	144 (54.5)	21 (65.6)	123 (53.0)	0.25
Stage, n (%)				
I/II	148	15	133	
IIIA	60	11	49	
IIIB/IIIC	56 (21.2)	6 (18.8)	50 (21.6)	0.894
Lymph node metastasis, n (%)				
N0	108	13	95	0.875
N1/2	98	13	85	0.808
N3	58 (22.0)	6 (18.8)	52 (22.4)	0.809
ECOG performance, n (%)				
0 or 1	231	28	203	
≥ 2	33 (12.5)	4 (12.5)	29 (12.5)	0.775
HER2-IHC status, n (%)				
IHC 3+ or 2+ plus FISH amplified	32 (12.1)	32	0	
IHC 2+ or 1+	33	0	33	
IHC 0	199	0	199	
Primary tumor, n (%)				
Stomach	244	31	213	
GEJ	20 (7.6)	1 (3.2)	19 (8.2)	0.483
Lauren histology type, n (%)				
Intestinal	123 (46.6)	24 (75.0)	99 (42.7)	0.001*
Diffuse	94	5	89	0.020*
Mixed	42	2	40	0.128
Others§	5	1	4	
H. pylori colonization, n (%)				
Yes	73 (27.7)	8 (25.0)	65 (28.0)	0.883
Microscopic or antigen test	64	7	57	
Culture isolate	9	1	8	
Serum CEA, ng/mL				
Median (IQR)	2.13 (1.35-3.57)	2.92 (1.51-5.60)	2.10 (1.32-3.39)	0.081
Serum CA19-9, U/mL				
Median (IQR)	14.67 (6.98-27.03)	16.49 (10.80-76.65)	14.10 (6.90-26.00)	0.034*
Recurrence or progression, n (%)				
Yes	121 (45.8)	11 (34.4)	110 (47.4)	0.23
Preoperative therapies, n (%)				
Yes	3	0	3	
CCRT	1		1	
Chemotherapy	2		2	
No	261 (98.9)	32 (100.0)	229 (98.7)	
HER2-directed therapy, n (%) $				
Yes	3	3 (9.4)	0	

Chemoradiotherapy (CCRT) or RT as salvage treatment (second- or third-line, CCRT, n=10; RT, n=1) while some had supportive care only (n=46 in total).

Survival outcome

In the median follow-up time of 31.2 months (95% CI 28.2-34.1 months), the 1 and 5-year RFS rate were stage I, 93.1/67.0%; stage II,

Table 2: Primary treatment for patients with early or locally advanced HER2-positive/negative gastric cancer.

	HER2-positive Primary treatment (n=32) n, (%)	HER2-negative Primary treatment (n=232) n, (%)	p
Operation only	18 (56.3)	113 (48.7)	0.541
Operation with adjuvant therapy	10 (31.3)	100 (43.1)	0.278
5-FU/platinum	5 (50.0)	32 (32.0)	
XELOX	3 (30.0)	18 (18.0)	
S-1	1 (10.0)	37 (37.0)	
Others*	1 (10.0)	13 (13.0)	
Systemic therapy**	1 (3.1)	8 (3.4)	0.98
HER2-directed therapy	0	0	ns
Supportive care	3 (9.4)	8 (3.4)	0.064
CCRT/RT	0	3 (1.3)	ns

*HER2-positive patients: Oral fluoropyrimidine (n=1); HER2-negative patients: oral fluoropyrimidine (n=11), FOLFOX (n=1) and IFL (n=1).
**HER2-positive patients: FOLFOX (n=1); HER2-negative patients: XELOX (n=3), fluoropyrimidine-based therapy (n=3), pembrolizumab (n=2) in the primary treatment. 5-FU: 5-fluorouracil; ns: Not Significant or comparable; FOLFOX: Folinic Acid, 5-fluorouracil and oxaliplatin; IFL: Folinic Acid and Infusional 5-fluorouracil; XELOX: Capecitabine and Oxaliplatin; ECF: Epirubicin, Cisplatin and 5-fluorouracil; S-1, gimeracil, oteracil and tegafur

93.0/60.4%; stage III, 59.7/22.6% and OS rate were stage I, 94.7/86.8%; stage II, 96.5/57.7%; stage III, 76.1/27.5%. Advanced disease stage was associated with significantly poor RFS and OS (median RFS/OS: stage I, 76.9/undefined; stage II, 66.2/66.7; stage III, 13.8/21.8 months, p<0.001/<0.001 for trend; (Figure 3A, 3B)). In addition, patients with lymph node metastasis (N0 *vs.* N1/2 *vs.* N3, median RFS/OS: 76.9/undefined *vs.* 66.1/66.2 *vs.* 13.1/27 months, p<0.001/0.001 for trend; (Figure 1A, 1B)), elevated serum CA199 (low *vs.* high, median RFS/OS: 70.8/undefined *vs.* 13.8/21.8 months, HR 0.34/0.26, p=0.001/<0.001; (Figure 1C, 1D)) and tumor vascular invasion (No *vs.* Yes, median RFS/OS: 73.4/undefined *vs.* 24.7/52.2 months, HR 0.47/0.33, p<0.001/<0.001; (Figure 2C, 2D)) had significantly poor RFS and OS. HER2 positivity was associated with a trend of shorter RFS but not statistically significant (HER2-(+) *vs.* (-), median RFS 18.2 *vs.* 54.0 months, HR=0.84, p=0.51; Figure 2A). However, patients with HER2-positive disease had a pronounced shorter OS as compared to negative patients (HER2-(+) *vs.* (-), median OS 30.0 *vs.* 79.2 months, HR=0.61, p=0.04; (Figure 2B)). In addition, *H. pylori* colonization did not correlate with an adverse survival outcome (Colonization no *vs.* yes, median RFS/OS: 43.1/79.2 *vs.* 59.9/undefined months, HR 0.88/0.88, p=0.5/0.6; (Supplementary 2A, 2B)).

Prognostic factors

In determining the prognostic factors for RFS, we found that poor performance status, CA199 >37 U/mL, lymph node metastasis, primary tumor at GEJ, CEA >5 ng/mL, vascular invasion and diffuse type were associated with poor RFS in univariate regression. Although HER2 positivity was not an independent prognostic factor for RFS, we still included HER2 status for further analysis owing to clinical relevance. In multivariate regression, only poor performance status, lymph node metastasis, high CA199 and primary tumor at GEJ were significant independent factors for poor RFS (Table 3). Furthermore, performance status, CA199, lymph node metastasis, primary tumor location, CEA, histology type, tumor differentiation and HER2 positivity were significant and relevant factors predicting OS in univariate regression. Following multivariate regression, only poor performance status, lymph node metastasis, high CA199 and vascular invasion were independent prognostic factors for OS in our cohort (Table 4).

Discussion

Our study presented a comprehensive overview and real-world evidence of clinical characteristics and treatment outcome in patients with Resectable HER2-positive gastric cancer. HER2 positivity was 12.1% in the early or locally advanced gastric cancer patients according to current diagnostic guidance and in scope with the published incidence range of 9.8% to 23.0% worldwide [12,15,18]. In the ToGA trial, the screening positivity for HER2 with IHC and FISH was 22.1% in total population and presented with a large variation across geographic region and ethnicity, with the highest prevalence of 33.2% in Australia and lowest of 5.9% in Taiwan [13]. Additional reports had confirmed the HER2 positivity of 6.1% to 10.4% in Han Chinese population incorporating a larger scale of screening and updated scoring system [11,12,16,19]. However, tumor heterogeneity, absence of universal screening in early diseases, inconsistent scoring and sampling limitations still contributed to discordance in the published HER2 prevalence. A routine evaluation of HER2 positivity in all gastric cancer patients would facilitate further investigation and ongoing clinical trials. In addition, HER2-positive patients correlated with male predominance, intestinal histology, higher CEA and CA199 as compared to negative patients in our cohort, compatible to previous reports [8,18,20]. However, we found more HER2-positive patients with primary tumor location at stomach but not GEJ. Although GEJ tumors are prevalent in western population and considered to be enriched in HER2 amplification, the discrepancy may result from the Asian ethnic background and delayed diagnosis of early proximal lesions [9,10,21].

Incorporation of trastuzumab with chemotherapy successfully extends overall survival from 11.1 to 13.8 months in advanced HER2-positive gastric cancer [13]. The therapeutic benefit is even more pronounced in the Asian subgroup confirmed by other trials [21,22]. Whether the treatment efficacy be extended to early or locally advanced diseases have been intensively investigated [23,24]. However, other HER2-directed agents, such as lapatinib, pertuzumab and trastuzumab emtansine that applied to breast cancer failed to demonstrate comparable benefits in HER2-positive gastric cancer, suggesting the role of HER2 pathways were distinctive in the two cancers and implied that "one size does not fit two" [22,25-27]. In our study, we found patients with HER2-positive disease significantly undertreated by HER2-directed agents with the target-drug exposure of less than 10% regardless in the frontline or recurrent condition. The therapeutic efficacy was rather obscure since the patients potentially benefited from HER2-directed therapies were deprived

Table 3: Prognostic factors for recurrence-free survival (RFS) in univariate and multivariate regression analyses.

Independent covariates	Univariate regression				Multivariate regression		
	n=	HR	95% CI	p	HR	95% CI	p
Performance							
ECOG 0 or 1	231	1					
ECOG ≥2	33	4.64	2.94-7.31	<0.001*	6.05	3.51-10.43	0.001*
Lymph node							
N0	108	1					
N1/2	98	1.56	1.01-2.40	0.049*	2	1.20-3.32	0.008*
N3	58	3.1	1.98-4.89	<0.001*	3.31	1.88-5.82	0.001*
CA199							
≤37 U/mL	230	1					
>37 U/mL	34	2.7	1.70-4.26	0.001*	2.07	1.26-3.39	0.004*
Primary tumor							
Stomach	244	1					
GE junction	20	2.57	1.49-4.43	0.001*	1.99	1.05-3.77	0.035*
Vascular invasion							
Yes	120	1					
No	144	0.5	0.33-0.76	0.001*	0.68	0.41-1.13	0.14
CEA							
≤5 ng/mL	225	1					
>5 ng/mL	39	1.78	1.13-2.73	0.012*	1.13	0.69-1.85	0.623
HER2 status							
Negative	232	1					
Positive	32	1.19	0.47-1.50	0.51	1.2	0.60-2.43	0.592
Histology							
Intestinal	123	1					
Diffuse	97	1.49	1.01-2.21	0.045*	1.18	0.76-1.84	0.462
Mixed	39	1.2	0.70-2.04	0.503	1.19	0.68-2.10	0.547
Differentiation							
Poor	166	1					
Well/Moderate	98	0.53	0.36-0.79	0.002*			
H. pylori colonization							
Yes	73						
No	191	1.14	0.77-3.40	0.507			
Sex							
Female	120	1					
Male	144	1.06	0.75-1.52	0.738			
Age	264	1.02	1.01-1.03	0.061			

of the intensive treatment. The real-world experience reflected that HER2-directed therapies may not be adequately accessible to patients with HER2-positive gastric cancer.

Comprehensive molecular evaluation of 295 primary gastric adenocarcinoma from The Cancer Genome Atlas (TCGA) revealed that a subset (49.8%) of tumors enriched in amplifications of receptor tyrosine kinases such as EGFR, HER2 and HER3 and featured by marked chromosomal instability [28]. Another cohort from Asian Cancer Research Group (ACRG) with whole-exome sequencing demonstrated a compatible molecular subgroup with microsatellite stability, inactivation in TP53, low tumor mutation and frequent amplifications in EGFR and HER2, also named as MSS/TP53-ve [29]. A series of reports confirmed that the molecular subtypes were prognostic, comparable to clinical observations such as HER2-positive diseases [30]. Indeed, HER2 positivity in gastric cancer had been debated as an independent poor prognostic factor. Jørgensen et al. indicated that HER2 positivity correlated with significantly poor survival and clinicopathological features in 30/42 published studies [15]. However, the prognostic role of HER2 status was seldom highlighted in resectable diseases. Some reports confirmed that HER2 positivity was associated with recurrence and inferior survival in Resectable gastric cancer [31]. Kanayama et al. proposed that HER2

Table 4: Prognostic factors for Overall-Survival (OS) in univariate and multivariate analyses.

Independent covariates	Univariate regression				Multivariate regression		
	n=	HR	95% CI	p	HR	95% CI	p
Performance							
ECOG 0 or 1	231	1					
ECOG ≥2	33	6.51	3.98-10.64	<0.001*	9.36	4.99-17.55	<0.001*
Lymph node							
N0	108	1					
N1/2	98	2.13	1.25-3.63	0.006*	2.34	1.26-4.37	0.007*
N3	58	3.62	2.10-6.25	<0.001*	2.98	1.54-5.78	0.001*
CA199							
≤37 U/mL	230	1					
>37 U/mL	34	3.11	1.90-5.08	<0.001*	2.09	1.23-3.57	0.007*
Vascular invasion							
Yes	120	1					
No	144	0.33	0.19-0.58	<0.001*	0.43	0.23-0.82	0.011*
CEA							
≤5 ng/mL	225	1					
>5 ng/mL	39	1.93	1.17-3.18	0.010*	1.16	0.65-2.09	0.621
Primary tumor							
Stomach	244	1					
GE junction	20	2.55	1.35-4.82	0.004*	1.73	0.82-3.67	0.151
Histology							
Intestinal	123	1					
Diffuse	97	1.69	1.06-2.68	0.026*	1.3	0.76-2.22	0.332
Mixed	39	1.18	0.63-2.21	0.603	1.13	0.58-2.22	0.723
HER2 status							
Negative	232	1					
Positive	32	1.65	1.01-3.08	0.040*	1.02	0.46-2.24	0.97
Differentiation							
Poor	166	1					
Well/Moderate	98	0.43	0.26-0.70	0.001*			
H. pylori colonization							
Yes	73	1					
No	191	1.14	0.72-1.79	0.588			
Sex							
Female	120	1					
Male	144	1.06	0.89-1.31	0.594			
Age	264	1.03	1.01-1.05	0.051			

amplification occurred early and involved in tumor progression in stage I gastric cancer [32]. However, we failed to identify a significant correlation of HER2 status with higher recurrence. We observed shorter RFS and OS in HER2-positive patients in scope with the published results, but the prognostic value diminished in multivariate analyses. More information and patients are required to consolidate the result and hence support the potential of HER2-directed therapies in early gastric cancer.

In addition to HER2 positivity, we found serum CA199, lymph node metastasis and tumor vascular invasion were independent factors for poor survival outcome. Serum CA199 could be considered as a surrogate marker for high tumor burden and also extensive lymph node involvement underlined an aggressive and disseminated disease. Our surgical outcome was similar with the Asian-based studies on D2 gastrectomy and enabled precise identification of lymph node metastasis [33,34]. In addition, vascular invasion correlated with depth, extent of tumor invasion and tendency of dissemination and therefore be recognized as an indicator for recurrence or inferior survival [35,36]. Tumor vascular invasion was reported to facilitate intratumoral angiogenesis, of which may serve as a potential target with anti-angiogenic agents [37,38].

There are several limitations in the study. First, it was a retrospective analysis incorporating patients treated in one institute and cannot address prospective therapeutic decisions and consequences. Second, we cannot eradicate selection bias and physician preferences for surgically-fit patients in our cohort. Third, patients with very early gastric cancer might not be evaluated for HER2 status and therefore were excluded from the study. However, our study still provided some real-world evidence and screening HER2 prevalence in Resectable gastric cancer. Since only few of HER2-positive patients had received HER2-directed therapies, our results can represent the nature disease course unaffected by HER2 targeted agents and be utilized to compare with future intervention trials.

Conclusion

We described the clinical characteristics and treatment outcome of patients with resectable HER2-positive gastric cancer. Curative operation with adjuvant chemotherapy remains the mainstay of primary treatment. HER2-positive patients are undertreated by HER2-directed therapies. HER2 positivity alone does not predict recurrence or progression but is associated with inferior overall survival. Poor performance status, lymph node metastasis and high CA199 predict a worse survival outcome. Ongoing prospective studies may provide information on the role of HER2-directed therapies in Resectable HER2-positive gastric cancer.

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Informed Consent and Ethical Statement

This is a retrospective observational study including patients with standard medical care. The study was approved by the institutional review board (IRB) with registry serial number B-ER-107-261 in National Cheng Kung University hospital, Tainan, Taiwan in accordance with the Declaration of Helsinki and its amendments.

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