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Evolution of HER2-low expression from primary to paired metastatic gastric cancer lesions

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HER2-low expression has recently gained considerable attention as an actionable biomarker in gastric cancer. However, changes in HER2-low expression between primary and metastatic gastric cancers remain inadequately explored. This study included consecutive patients diagnosed with metastatic gastric cancer with both primary and metastatic tumors, between January 2014 and December 2023. HER2 status was evaluated in both primary and matched metastatic tumors. A total of 332 patients were enrolled, with HER2-negative, HER2-low, and HER2-positive statuses were observed in 226, 81, and 25 primary tumors, respectively, and in 175, 104, and 53 metastatic tumors, respectively. Among the 226 patients with HER2-negative primary tumors, 74 and 23 developed HER2-low and HER2-positive metastatic tumors, respectively. Conversion from HER2-negative primary to HER2-low metastatic gastric cancer was associated with metachronous and non-peritoneal metastasis. Overall, re-biopsy to evaluate HER2 status may be necessary, potentially broadening the patient population eligible for targeted HER2 therapy.

HER2, an oncogene, contributes significantly to gastric cancer development[1]. HER2 dimerization activates various signaling pathways that lead to tumor cell proliferation and invasive growth[2]. HER2 status is classified as either positive or negative based on immunohistochemistry (IHC) scores. HER2-positive is defined as an IHC score of 3+ or 2+ with HER2 gene amplification[3]. HER2-negative is defined as IHC score of 0, 1+, or 2+ without HER2 gene amplification[3].

In patients with HER2-positive advanced gastric cancer, the combination of trastuzumab and chemotherapy significantly improves overall survival (OS) compared to chemotherapy alone (median OS, 13.8 months vs. 11.1 months, p = 0.046)[4]. This regimen has been the standard treatment for over a decade[5]. Additionally, the KEYNOTE-811 study demonstrated that the addition of pembrolizumab to the chemotherapy and trastuzumab combination significantly improved the objective response rate and progression-free survival (PFS) compared with chemotherapy and trastuzumab alone (median PFS, 10.0 months vs. 8.1 months; p = 0·0002)[6]. The combination of programmed cell death protein 1 (PD-1) and HER2 blockade with chemotherapy could significantly improve the objective response rate and emerge as first-line treatment in patients with HER2-positive advanced gastric cancer. However, HER2 positivity occurs is present in only 7–20% of gastric cancers[7], highlighting the need for new effective treatments for the majority of gastric cancer cases that are HER2-negative.

HER2-low gastric cancers have recently emerged as a distinct subgroup[8]. HER2-low is defined as a HER2 IHC 1+ or 2+, without HER2 gene amplification[8]. Previously, HER2-low gastric cancer was considered HER2-negative, and patients with this subtype were not expected to benefit from trastuzumab therapy. However, trastuzumab deruxtecan (T-DXd; DS-8201), an antibody-drug conjugate, has demonstrated promising efficacy against HER2-low tumors. In an exploratory cohort of a phase II trial, 17.5% (7/40) of patients with HER2-low gastric cancer achieved a partial response to T-DXd. The median OS was 7.8 months for patients with HER2 IHC 2+ without HER2 gene amplification, and 8.5 months for patients with HER2 IHC 1+ gastric cancer[9]. In addition to T-DXd, other therapies, including

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Disitamab vedotin, ARX788, LCB-ADC, and PF-0680410[10–13] are effective against HER2-low tumors. These therapies offer the potential to prolong the survival of patients with HER2-low gastric cancer.

HER2 expression in gastric cancer can be heterogeneous between primary and distant metastatic sites. For instance, patients with HER2-negative primary gastric cancer may develop HER2-positive metastatic tumors[14]. However, previous studies have typically classified HER2 expression as either negative or positive[15]. Consequently, the dynamics of HER2-low expression in primary versus distant metastatic gastric cancers remain largely unknown. This gap in understanding is clinically significant, as metastatic tumors may provide a more comprehensive "predictive window" than primary tumors alone. Uzunparmak et al. evaluated HER2 status in both primary and metastatic tumors from 25 patients with advanced gastric cancer[16] and found that none of the patients with HER2-negative primary gastric cancers developed HER2-low metastatic tumors[16]. In contrast, a study by Bozzetti et al., which evaluated 18 patients with HER2 IHC 0 primary gastric cancer, revealed that three of these patients had HER2 IHC 1+ metastatic tumors[17]. However, both studies were limited by small sample sizes, which restricts the ability to draw definitive conclusions.

Therefore, in this study, we aimed to evaluate the evolution of HER2 status between primary and distant metastatic tumors in gastric cancer and categorized HER2 status into three groups: HER2-negative (IHC 0), HER2-low (IHC 1+ and 2+ without gene amplification), and HER2-positive (IHC 3+ and 2+ with gene amplification).

Results

Patient characteristics

A total of 332 patients with a median age of 55 years at diagnosis were included in this study (Fig. 1). Among the enrolled patients, 187 (56.3%) were male and 145 (43.7%) were female. Metastases were metachronous and synchronous in 89 (26.8%) and 243 (73.2%) patients, respectively. For patients with metachronous metastasis, primary tumor samples were collected before metastatic diagnosis. In patients with synchronous metastasis, biopsies of both the primary tumor and metastatic lesions were performed. Of the 332 patients, 226 (68.1%), 81 (24.4%), and 25 (7.5%) patients had HER2-negative, HER2-low, and HER2-positive primary gastric cancers, respectively. Metastatic lesions were primarily observed in the peritoneum (n = 201), ovaries (n = 49), liver (n = 23), lungs (n = 9), distant lymph nodes (n = 9), and brain (n = 4), with 37 cases occurring in other sites. Metastatic tumors were obtained via surgical procedures (n = 292) and biopsies (n = 40). Among the patients undergoing surgical sampling, 201 had peritoneal metastasis, 49 had ovarian metastasis, 20 had liver metastasis, 9 had lung metastasis, 5 had diaphragmatic metastasis, 4 had brain metastasis, and 4 had distant lymph node metastasis. The clinical characteristics of patients are presented in Table 1.

A total of 175 (52.7%), 104 (31.3%), and 53 (16.0%) patients had HER2-negative, HER2-low, and HER2-positive metastatic gastric cancers, respectively (Supplementary Table 1). Compared with HER2-negative metastatic tumors, HER2-low metastatic gastric cancers were more highly associated with metachronous metastasis and fewer instances of peritoneal metastasis. Similarly, HER2-positive metastatic gastric cancers also showed fewer occurrences of peritoneal metastasis. HER2-low and HER2-positive metastatic gastric cancers exhibited similar features, with the notable difference that a higher proportion of patients with HER2-low metastatic gastric cancer were aged over 60 years.

Evolution of HER2 expression from primary to metastatic gastric cancer

The evolution of HER2 expression from primary to metastatic gastric cancer is depicted in Fig. 2. The overall HER2 discordance rate was 48.8% (162/332). Specifically, 111 (33.4%) patients experienced an upward shift in HER2 expression, while 51 (15.4%) patients experienced a downward shift. The Cohen's kappa coefficient for HER2 expression was 0.120. Among the 226 patients with HER2-negative primary tumors, 74 (32.7%) and 23 (10.2%) developed HER2-low and HER2-positive metastatic tumors, respectively. The conversion from HER2-negative primary cancer to HER2-low metastatic cancer was associated with metachronous and non-peritoneal metastases (Table 2).

Among the 81 patients with HER2-low primary gastric cancer, 42 (51.9%) developed HER2-negative metastatic tumors. The conversion from HER2-low primary cancer to HER-negative metastatic cancer was associated with peritoneal metastasis (Supplementary Table 2). Additionally, 14 (17.3%) patients with HER2-low primary gastric cancer developed HER2-positive metastatic tumors (Fig. 2).

The HER2-positive phenotype exhibited the highest stability. Among the 25 patients with HER2-positive primary gastric cancer, 16 (64%) had HER2-positive metastatic tumors, 5 (20%) had HER2-low metastatic tumors, and only 4 (16%) had HER2-negative metastatic tumors (Fig. 2).

Among the 201 patients with peritoneal metastasis, 130 (64.7%), 51 (25.4%), and 20 (10.0%) had HER2-negative, HER2-low, and HER2-positive metastatic gastric cancers, respectively. Among the 131 patients with non-peritoneal metastasis, 45 (34.4%), 53 (40.5%), and 33 (25.2%) had HER2-negative, HER2-low, and HER2-positive metastatic gastric cancers, respectively. Peritoneal metastasis was associated with HER2-negative status (Supplementary Table 3). Among the 139 patients with HER2-negative primary tumors who developed peritoneal metastasis, 96 (69.1%) had

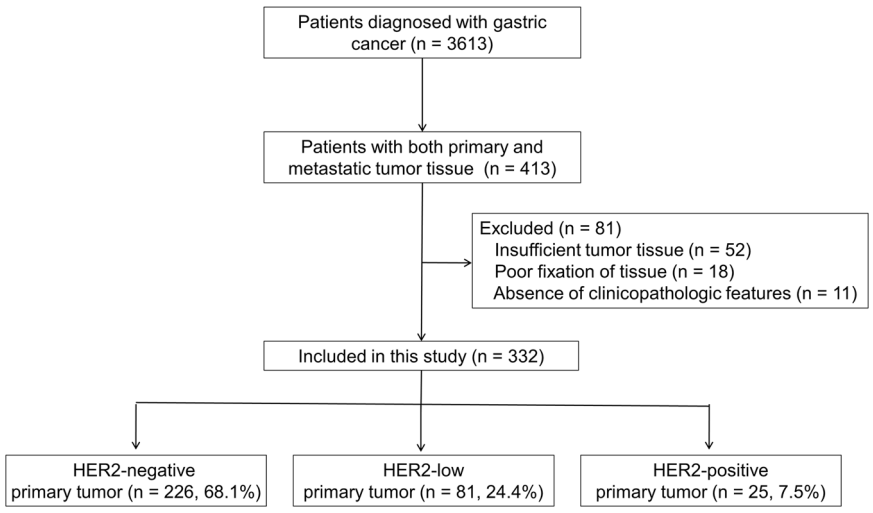


Fig. 1 | Patient selection flow chart.

Table 1 | Clinicopathological features of HER-2-negative, HER-2 low, and HER-2 positive primary gastric cancer

	Negative (%)	Low (%)	Positive (%)	P[a] (Neg vs. Low)	P[a] (Neg vs. Pos)	P[a] (Low vs. Pos)
Age				0.348	0.515	0.247
<60	146 (64.6)	47 (58.0)	18 (72.0)			
≥60	80 (35.4)	34 (42.0)	7 (28.0)			
Sex				0.150	0.673	0.166
Male	123 (54.4)	52 (64.2)	12 (48.0)			
Female	103 (45.6)	29 (35.8)	13 (52.0)			
Primary tumor site				0.675	0.216	0.399
Gastroesophageal junction/cardia/fundus	55 (24.3)	20 (24.7)	3 (12.0)			
Body	67 (29.6)	20 (24.7)	6 (24.0)			
Antrum	104 (46.0)	41 (50.6)	16 (64.0)			
Lauren classification				0.108	**0.024**	0.444
Diffuse	125 (55.3)	34 (42.0)	7 (28.0)			
Intestinal	39 (17.3)	23 (28.4)	10 (40.0)			
Mixed	56 (24.8)	21 (25.9)	8 (32.0)			
Not classified	6 (2.7)	3 (3.7)	0 (0)			
T				0.631	0.242	0.126
1 + 2	14 (6.2)	4 (5.0)	3 (12.0)			
3	117 (51.8)	47 (58.0)	9 (36.0)			
4	95 (42.0)	30 (37.0)	13 (52.0)			
N				0.474	0.442	0.229
0	20 (8.8)	11 (13.6)	0 (0)			
1	39 (17.3)	10 (12.3)	4 (16.0)			
2	39 (17.3)	12 (14.8)	4 (16.0)			
3	128 (56.6)	48 (59.3)	17 (68.0)			
Metastases				0.664	1.000	0.795
Synchronous	164 (72.6)	61 (75.3)	18 (72.0)			
Metachronous	62 (27.4)	20 (24.7)	7 (28.0)			
Metastatic site				0.226	0.250	0.371
Peritoneum	139 (61.5)	50 (61.7)	12 (48.0)			
Liver	15 (6.6)	4 (4.9)	4 (16.0)			
Ovarian	35 (15.5)	9 (11.1)	5 (20.0)			
Lung	8 (3.5)	1 (1.2)	0 (0)			
Distant lymph node	3 (1.3)	5 (6.2)	1 (4.0)			
Brain	2 (0.9)	1 (1.2)	1 (0)			
Other	24 (10.6)	11 (13.6)	2 (8.0)			

[a]Differences were analyzed with the chi-square test for sample sizes ≥5, and Fisher's exact test for sample sizes <5.
The bold values mean $p < 0.05$.

HER2-negative metastatic tumors, whereas 35 (25.2%) and 8 (5.8%) had HER2-low and HER2-positive metastatic tumors, respectively (Fig. 3A). Among the 87 patients with HER2-negative primary tumors who developed non-peritoneal metastasis, 33 (37.9%) had HER2-negative metastatic tumors. Notably, 39 (44.8%) had HER2-low metastatic tumors, and 15 (17.2%) had HER2-positive metastatic tumors (Fig. 3B).

Among the 49 patients with ovarian metastasis, 35 (71.4%), 9 (18.4%), and 5 (10.2%) had HER2-negative, HER2-low, and HER2-positive primary cancers, respectively. In contrast, 10 (20.4%), 24 (49.0%), and 15 (30.6%) had HER2-negative, HER2-low, and HER2-positive metastatic cancers, respectively (Fig. 4A). Among the 23 patients with liver metastasis, 15 (65.2%), 4 (17.4%), and 4 (17.4%) had HER2-negative, HER2-low, and HER2-positive primary cancers, respectively, whereas 8 (34.8%), 10 (43.5%), and 5 (21.7%) had HER2-negative, HER2-low, and HER2-positive metastatic cancers, respectively (Fig. 4B).

Besides evaluating metastatic sites as binary variables, we also analyzed them in a combinatorial manner. Among the 32 patients with liver or lung metastasis, 23 (71.9%), 5 (15.6%), and 4 (12.5%) had HER2-negative, HER2-low, and HER2-positive primary cancers, respectively. In comparison, 9 (28.1%), 17 (53.1%), and 6 (18.8%) had HER2-negative, HER2-low, and HER2-positive metastatic cancers, respectively (Supplementary Fig. 1). Among the 27 patients with liver or brain metastasis, 17 (63.0%), 5 (18.5%), and 5 (18.5%) had HER2-negative, HER2-low, and HER2-positive primary cancers, respectively, whereas 9 (33.3%), 10 (37.0%), and 8 (29.6%) had HER2-negative, HER2-low, and HER2-positive metastatic cancers, respectively (Supplementary Fig. 2).

Fifteen patients had matched samples from both primary and metastatic tumors from two different organ sites. Among the nine patients with HER2-negative primary tumors, seven developed HER2-expressing metastatic tumors, while two had HER2-negative metastatic tumors (Supplementary Table 4).

Fig. 2 | Sankey diagrams illustrating HER2 status between primary and metastatic gastric cancers.

		HER2 Primary n, %			Total
		HER2-negative	HER2-low	HER2-positive	
HER2 Metastasis n, %	HER2-negative	129 (57.1)	42 (51.9)	4 (16.0)	175
	HER2-low	74 (32.7)	25 (30.9)	5 (20.0)	104
	HER2-positive	23 (10.2)	14 (17.3)	16 (64.0)	53
Total		226	81	25	332

Response of one patient with HER2-negative primary and HER2-low metastatic gastric cancer to anti-HER2 treatment

The patient, a 71-year-old woman, underwent surgery on January 9, 2020, for stage IIIA (pT3N2M0) gastric cancer, with the primary tumor identified as HER2-negative. In August 2021, follow-up imaging revealed metastasis in the supraclavicular and superior mesenteric paravenous lymph nodes. A supraclavicular lymph node puncture confirmed metastatic gastric cancer, showing HER2 IHC 2+ without HER2 gene amplification. The patient began first-line treatment with oxaliplatin, capecitabine, and sintilimab in August 2021. After 12 months, the cancer progressed, and paclitaxel was initiated as second-line therapy. By May 2023, the cancer progressed further, with new liver metastasis emerging. The treatment regimen was then switched to systemic therapy, with Disitamab vedotin plus toripalimab as third-line treatment. The metastatic tumors responded, showing shrinkage that persisted for approximately six months (Supplementary Fig. 3).

Treatment outcome of patients who were with HER2-positive metastatic cancer

Among the 53 patients with HER2-positive metastatic gastric cancer, 23 had HER2-negative, 14 had HER2-low, and 16 had HER2-positive primary tumors. For the 23 patients with HER2-negative primary and HER2-positive metastatic gastric cancer, 10 patients received oxaliplatin-based (FOLFOX/XELOX/SOX) chemotherapy as first-line treatment, while 12 patients received oxaliplatin-based (FOLFOX/XELOX/SOX) chemotherapy plus PD-1 antibody as first-line treatment. One patient with HER2-negative primary and HER2-positive metastatic gastric cancer was treated with oxaliplatin, 5-Fu, trastuzumab plus pembrolizumab as first-line treatment, progression-free survival lasting more than 20 months. For the 14 patients with HER2-low primary and HER2-positive metastatic gastric cancer, 9 received oxaliplatin-based (XELOX/SOX) chemotherapy as first-line treatment, and 5 received oxaliplatin-based (XELOX/SOX) chemotherapy plus PD-1 antibody as first-line treatment. There was no significant difference in OS among patients with HER2-negative, HER2-low, or HER2-positive primary tumors in those with HER2-positive metastatic gastric cancer ($p = 0.531$, log-rank test), as shown in the Supplementary Fig. 4 (median overall survival was 20.3, 18.2, and 21.0 months, respectively).

Discussion

In this study, we investigated the concordance of HER2 status between primary and corresponding metastatic gastric cancers, incorporating HER2-low expression as a subgroup. HER2-low expression was observed in 24.4% of primary gastric tumors and 31.3% of metastatic tumors. Notably, 32.7% (74/226) of patients with HER2-negative primary gastric cancer developed HER2-low metastatic tumors. Among patients without peritoneal metastasis, 44.8% (39/87) of those with HER2-negative primary gastric cancer developed HER2-low metastatic tumors. To the best of our knowledge, this research represents the largest study to date on the evolution of HER2-low expression from primary to distant metastatic gastric cancers.

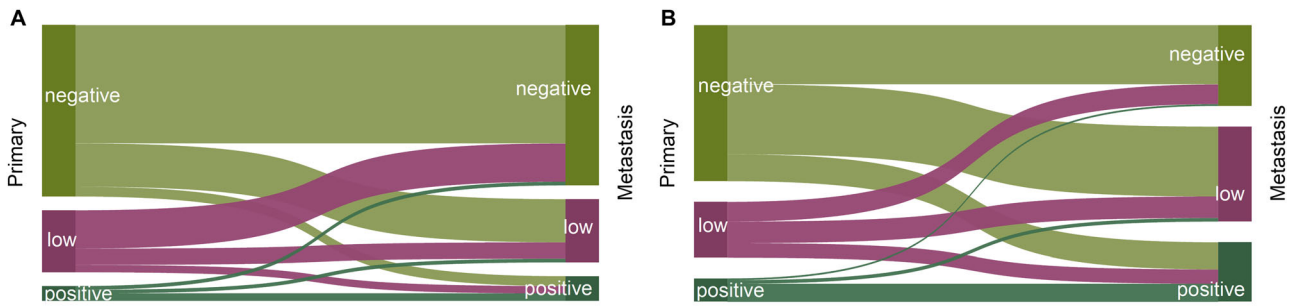
Our observations align with those of previous studies reporting a higher HER2 positivity rate in metastases than in primary lesions. Fusco et al. assessed HER2 expression in primary cancers and lymph node metastases, identifying six patients with HER2-negative primary gastric cancer who had HER-2 positive metastatic lymph nodes[14]. Similarly, Park et al. evaluated the HER2 status of metastatic tumors in 175 patients with HER2-negative primary gastric cancer and observed a 5.7% conversion rate to HER2-positive in 10 patients[18]. In contrast, some studies have reported high concordance rates between primary and metastatic HER2 status. Bozzetti et al. evaluated HER2 status in both primary and metastatic tumors in 72 patients with gastric cancer, observing 11 HER2-positive metastatic tumors and 10 HER2-positive primary tumors[17]. Previous studies have classified HER2 status as negative (IHC 0, 1+, or 2+ without HER2 gene amplification) or positive (IHC 3+ or 2+ with HER2 gene amplification). However, in this study, we introduced HER2-low expression as a distinct category. Our results highlight the clinical importance of re-biopsying metastasis, as patients with HER2-negative primary gastric cancer are likely to develop HER2-low metastatic tumors, which may respond new generations of HER2-targeted therapies. The genomic differences between primary and metastatic tumors are well recognized. A large study, which included sequencing data from 25,755 patients, evaluated genomic mutations associated with metastasis to specific organs. Among these patients, 160 with gastric cancer were included, and the metastatic tumors exhibited a higher tumor mutation burden and more actionable mutations than the primary tumors[19]. These findings, along with our observations, indicate that metastatic tumors may serve as a better "predictive window" for targeted therapy.

A previous study suggested that anti-HER2 therapy may alter HER-2 expression status[20]. In the present study, 243 patients (73.2%) presented with synchronous metastasis, with samples collected before any treatment. For the remaining 89 patients (26.8%) with metachronous metastasis, metastatic tumor samples were obtained prior to

Table 2 | HER-2 status of metastatic tumors in patients with HER-2 negative primary gastric cancer

	Negative (%)	Low (%)	Positive (%)	P[a] (Neg vs. Low)	P[a] (Neg vs. Pos)	P[a] (Low vs. Pos)
Age				0.652	**0.031**	**0.022**
<60	82 (63.6)	44 (59.5)	20 (87.0)			
≥60	47 (36.4)	30 (40.5)	3 (13.0)			
Sex				0.185	**0.038**	0.237
Male	78 (60.5)	37 (50.0)	8 (34.8)			
Female	51 (39.5)	37 (50.0)	15 (65.2)			
Primary tumor site				0.576	0.789	0.503
Gastroesophageal junction/cardia/fundus	30 (23.3)	19 (25.7)	6 (26.1)			
Body	37 (28.7)	25 (33.8)	5 (21.7)			
Antrum	62 (48.1)	30 (40.5)	12 (52.2)			
Lauren classification				0.375	0.708	0.958
Diffuse	78 (60.5)	36 (48.6)	11 (47.8)			
Intestinal	21 (16.3)	13 (17.6)	5 (21.7)			
Mixed	27 (20.9)	23 (31.1)	6 (26.1)			
Not classified	3 (2.3)	2 (2.7)	1 (4.3)			
Metastases				**0.032**	0.420	0.803
Synchronous	101 (78.3)	47 (63.5)	16 (69.6)			
Metachronous	28 (21.7)	27 (36.5)	7 (30.4)			
Metastatic site				**<0.001**	**<0.001**	0.361
Peritoneum	96 (74.4)	35 (47.3)	8 (34.9)			
Liver	6 (4.7)	7 (9.5)	2 (8.7)			
Ovarian	8 (6.2)	18 (24.3)	9 (39.1)			
Lung	1 (0.8)	6 (8.1)	1 (4.3)			
Distant lymph node	3 (2.3)	0 (0)	0 (0)			
Brain	1 (0.8)	0 (0)	1 (4.3)			
Other	14 (10.9)	8 (10.8)	2 (8.7)			
Source of metastatic tumor				0.053	**0.045**	0.335
Biopsy	21 (16.3)	5 (6.8)	0 (0)			
Surgery	108 (83.7)	69 (93.2)	23 (100)			

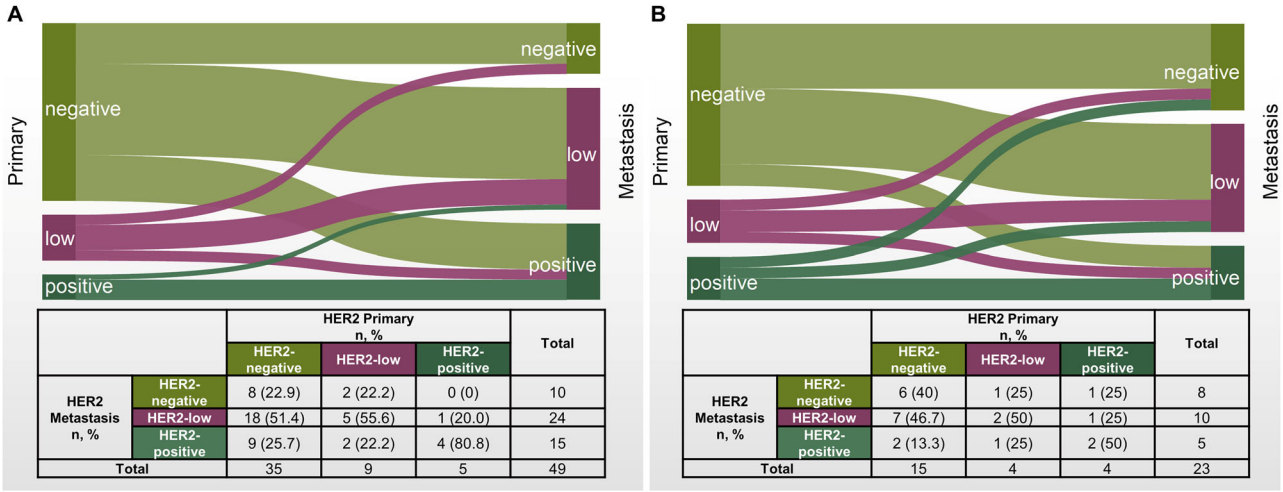
[a]Differences were analyzed with the chi-square test for sample sizes ≥5, and Fisher's exact test for sample sizes <5.
The bold values mean $p < 0.05$.



		HER2 Primary n, %			Total
		HER2-negative	HER2-low	HER2-positive	
HER2 Metastasis n, %	HER2-negative	96 (69.1)	31 (62.0)	3 (25.0)	130
	HER2-low	35 (25.2)	13 (26.0)	3 (25.0)	51
	HER2-positive	8 (5.8)	6 (12.0)	6 (50.0)	20
Total		139	50	12	201

		HER2 Primary n, %			Total
		HER2-negative	HER2-low	HER2-positive	
HER2 Metastasis n, %	HER2-negative	33 (37.9)	11 (35.5)	1 (7.7)	45
	HER2-low	39 (44.8)	12 (38.7)	2 (15.4)	53
	HER2-positive	15 (17.2)	8 (25.8)	10 (76.9)	33
Total		87	31	13	131

Fig. 3 | Evolution of HER2 expression from primary to metastatic tumors in patients with gastric cancer. Evolution of HER2 expression in patients with peritoneal metastasis (**A**) and non-peritoneal metastasis (**B**). The metastatic samples were biopsied from the corresponding metastatic sites.



A

		HER2 Primary n, %			Total
		HER2-negative	HER2-low	HER2-positive	
HER2 Metastasis n, %	HER2-negative	8 (22.9)	2 (22.2)	0 (0)	10
	HER2-low	18 (51.4)	5 (55.6)	1 (20.0)	24
	HER2-positive	9 (25.7)	2 (22.2)	4 (80.8)	15
Total		35	9	5	49

B

		HER2 Primary n, %			Total
		HER2-negative	HER2-low	HER2-positive	
HER2 Metastasis n, %	HER2-negative	6 (40)	1 (25)	1 (25)	8
	HER2-low	7 (46.7)	2 (50)	1 (25)	10
	HER2-positive	2 (13.3)	1 (25)	2 (50)	5
Total		15	4	4	23

Fig. 4 | Evolution of HER2 expression from primary to metastatic tumors. Evolution of HER2 expression in patients with gastric cancer with ovarian metastasis (**A**) and liver metastasis (**B**).

initiating palliative chemotherapy. Importantly, none of the patients had received anti-HER2 therapy before metastasis sampling.

Another notable finding is the association between the evolution of HER2-low expression and the site of metastasis. Among patients with HER2-negative primary tumors who developed peritoneal metastasis, 69.1% of metastatic tumors were HER2-negative, while only 25.2% were HER2-low. In contrast, among the 131 patients with HER2-negative primary tumors who developed non-peritoneal metastasis, 37.9% of metastatic tumors were HER2-negative, while 44.8% were HER2-low. Consistently, previous studies have also highlighted a correlation between the absence of peritoneal metastasis and HER2 positivity[21]. Saito et al. assessed HER2 status in 52 patients with gastric cancer liver metastasis and 85 patients with peritoneal metastasis, finding a lower HER2 positivity rate in peritoneal metastasis compared to liver metastasis[22]. These results suggest a negative association between peritoneal metastasis and HER2 positivity. Furthermore, peritoneal metastasis has been linked to reduced efficacy of HER2-targeted therapy. In a cohort of 24 patients with HER2-positive gastric cancer receiving combination therapy with trastuzumab, those with peritoneal metastasis had a shorter PFS compared to those without peritoneal metastasis[23]. However, the exact mechanism underlying this adverse relationship remains unclear. With advancements in sequencing techniques, researchers have begun to explore the molecular characteristics of peritoneal metastasis. Wang et al. performed whole-exome and whole-transcriptome sequencing on a cohort of 43 patients with gastric cancer and peritoneal metastasis, observing distinct mutation patterns in peritoneal metastatic tumors[24]. Specifically, *CDH1* exhibited a 3-fold higher mutation rate in peritoneal metastasis compared to primary gastric cancer[24]. Additionally, Tanaka et al. reported a higher frequency of alterations in the RTK and MAPK pathways in peritoneal metastasis than in primary gastric cancer[25]. Recently, Zhao et al. conducted a multi-omic study involving 326 patients and found that molecular aberrations, such as *TP53*, *ARID1A*, *CDH1* and *PIK3CA* mutations, were associated with peritoneal metastasis. In addition, the transcriptomic profile of peritoneal metastasis was distinct from that of liver metastasis[26]. Using single-cell sequencing technology, Wang et al. analyzed the intratumoral heterogeneity of peritoneal metastasis and found that tumor cell lineage compositions were associated with survival outcomes in patients with gastric cancer[27]. Furthermore, immune cells in peritoneal metastasis also exhibited distinct phenotypes and compositions[28]. These results suggest a unique mutational landscape for peritoneal metastasis.

Intratumoral HER2 heterogeneity has been recognized as a relevant factor in gastric cancer. However, there is currently no universally accepted definition of heterogeneity. In a study of 875 gastric cancer cases, Nishida et al. defined intratumoral heterogeneity as different IHC scores between two separate tissue cores[29]. Hofmann et al. considered heterogeneity to be present when <10% of tumor cells stained positive[30], while Cutsem et al. used a threshold of <30%[31]. Moreover, intratumoral heterogeneity was more frequently observed in patients with HER2 IHC 2+ primary gastric cancer and was rarely reported in patients with HER2 IHC 0 primary gastric cancer. In the present study, 32.7% (74/226) of patients with HER2-negative primary gastric cancer developed HER2-low metastatic tumors. We believe that the difference between primary and metastatic gastric cancer in our cohort is unlikely to be caused by intratumoral heterogeneity.

This study had several limitations. First, it was retrospective and conducted at a single institution. Second, detailed sampling conditions, such as fixation time, type of fixative, time before fixation, and formalin concentration, which could potentially influence the results of HER2 staining[32], were not considered. Third, while we identified patients with HER2-negative primary and HER2-low metastatic gastric cancers, the efficacy of anti-HER2 therapy in these patients remains unknown. In an exploratory cohort of the DESTINY-Gastric01 trial, 17.5% (7/40) of patients with HER2-low gastric cancer achieved a partial response to T-DXd[9]. Additionally, in a phase 1 trial, 35.3% (6/17) of patients with HER2-low gastric cancer responded to disitamab vedotin plus toripalimab[10]. Further research is needed to evaluate the efficacy of anti-HER2 therapy in patients with HER2-negative primary and HER2-low metastatic gastric cancer, as well as those with HER2-low primary and HER2-negative metastatic gastric cancer. Fourth, the inclusion of patients who received various systemic therapies limits the ability to definitively determine survival outcomes. Finally, this study did not compare other molecular biomarkers between primary and paired metastatic gastric cancers.

In conclusion, this study identified a significant proportion of patients with HER2-negative primary and HER2-low metastatic gastric cancers, particularly among those without peritoneal metastasis. A re-biopsy to evaluate HER2 status may be warranted, as it could expand the patient population eligible for targeted HER2 therapy. Further multicenter studies involving larger cohorts are needed to validate the findings of this study.

Methods

Patients

Consecutive patients diagnosed with metastatic gastric cancer who had both primary and metastatic tumors, and were treated at our institution between January 2014 and December 2023, were included in this study. Exclusion criteria were as follows: insufficient tumor tissue, poor tissue fixation, and absence of clinicopathologic features. This study was performed in accordance with the Declaration of Helsinki, and approved by the Ethics Review Board of Sun Yat-sen University Cancer Center (SL-B2023-585-01). Informed consent was obtained from all patients.

Immunohistochemistry-based assessment of HER2 expression

Formalin-fixed, paraffin-embedded tissues from both primary and metastatic tumors were selected for each patient. HER2 expression was assessed using the Ventana anti-HER2/neu (4B5) rabbit monoclonal primary antibody (Roche Diagnostics). Staining was performed on a Ventana Benchmark XT automatic staining system, according to the manufacturer's instructions. HER2 scoring was conducted according to the guidelines of the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology[33].

HER2 fluorescence in situ hybridization (FISH)

HER2 amplification in IHC 2+ tumors was assessed using the ZytoLight FISH-Tissue Implementation Kit, which includes a Spectrum Green fluorophore-labeled DNA probe for the HER2 gene locus and a Spectrum Orange fluorophore-labeled α-satellite DNA probe for chromosome 17 (SPEC HER2/CEN 17 Dual Color Probe Kit, ZytoVision). Hybridization was performed using the Hybrite denaturation/hybridization system. Images were analyzed using an OlympusMX60 fluorescence microscope (Olympus, Hamburg, Germany). HER2 gene amplification was defined by a HER2/CEN17 ratio of ≥2.0 or the presence of a HER2 cluster signal.

Definition of HER2 status

HER2 status was categorized as follows: HER2-negative (IHC 0), HER2-low (IHC 1+ or IHC 2+ without HER2 gene amplification), and HER2-positive (IHC 3+ or IHC 2+ with HER2 gene amplification).

Statistical analysis

All statistical analyses were performed using SPSS version 22.0 software (Statistical Product and Service Solutions, Chicago City, Illinois State, United States). Frequencies and descriptive statistics were used to summarize patient characteristics, while differences between groups were analyzed using the chi-square test. Cohen's kappa coefficient was used to evaluate the consistency between two trichotomous variables (HER2-negative, HER2-low, and HER2-positive). OS was defined as the time from metastasis diagnosis to the date of death or last follow-up (October 31, 2024). Survival curves were generated using the Kaplan-Meier method, and differences were assessed using the log-rank test. A P-value ≤ 0.05 was considered statistically significant.

Data availability

All data supporting the study findings are available upon reasonable request from the corresponding author.

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Competing interests

The authors declare no competing interests.

Additional information

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