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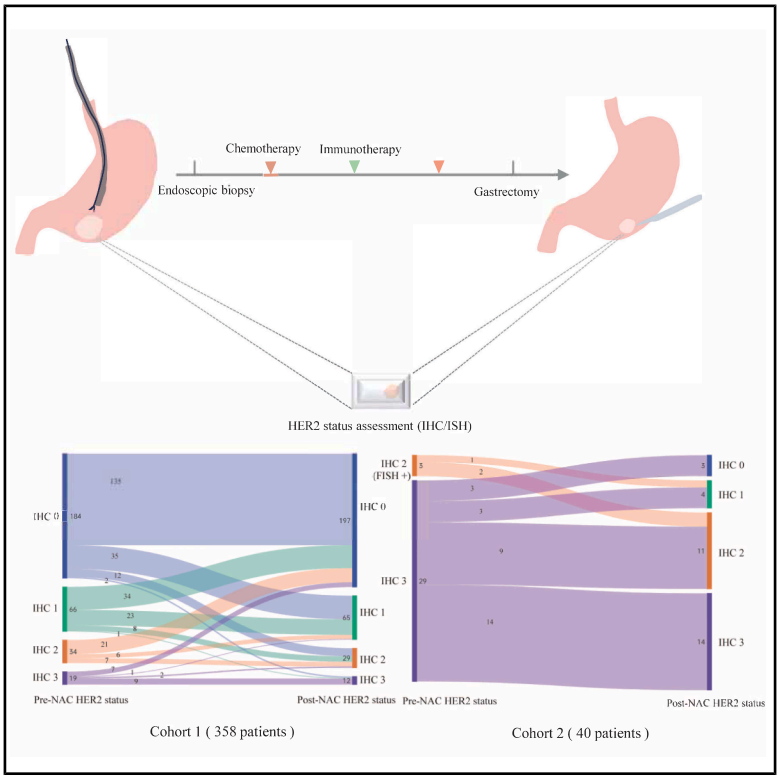
Evolution of HER2 expression after neoadjuvant therapy in locally advanced gastric cancer

Graphical abstract



Highlights

- Alterations in HER2 expression after NAT were detected in more than 40% of patients
- Decreased HER2 expression was associated with better prognosis
- Both PD-1/PD-L1 inhibitors and trastuzumab increased pCR rates

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In brief

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Evolution of HER2 expression after neoadjuvant therapy in locally advanced gastric cancer

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SUMMARY

HER2 is a crucial biomarker in gastric cancer, influencing both treatment strategies and patient prognosis. A multicenter retrospective study was conducted, including 398 patients with locally advanced gastric cancer who received neoadjuvant treatment (NAT) followed by gastrectomy between 2018 and 2023 at three medical centers in China. Alterations in HER2 expression after NAT were detected in more than 40% of patients, with a higher rate of decreased expression (26.0%) compared to increased expression (17.3%). Multivariate analysis indicated that HER2 status at diagnosis significantly influenced HER2 expression alteration. Patients with HER2 IHC 2+ tumors before NAT demonstrated an increased tendency for HER2 expression alterations after NAT. Decreased HER2 expression was associated with improved recurrence-free survival and overall survival. PD-1/PD-L1 inhibitors and trastuzumab both increased pCR rates, but neither significantly impacted the rate of HER2 expression alterations among non-pCR patients. Reassessing HER2 status after NAT is essential for guiding HER2-targeted therapies.

INTRODUCTION

Gastric cancer is a common gastrointestinal malignancy, with most patients being diagnosed at a locally advanced stage.[1,2] Neoadjuvant treatment (NAT) refers to all preoperative treatments, including chemotherapy, immunotherapy, and targeted therapy, while neoadjuvant chemotherapy (NAC) specifically refers to preoperative chemotherapy. Currently, NAC is a standard treatment option for locally advanced gastric cancer. The MAGIC study and the RESOLVE study both demonstrated that perioperative chemotherapy improves overall survival (OS) rates in patients with locally advanced gastric cancer.[3,4] Immunotherapy has been proven to improve survival outcomes in advanced gastric cancer, and its use in NAT for locally advanced gastric cancer is being actively explored.[5–8] Studies have revealed that adding immunotherapy to NAT can enhance the pathological complete response (pCR) rate in patients with locally advanced gastric cancer.[9,10]

The human epidermal growth factor receptor 2 (HER2) is an important biomarker in both breast and gastric cancer. Approximately 10% of gastric cancer patients are HER2-positive.[11,12] The ToGA study demonstrated that combining trastuzumab with chemotherapy improves survival outcomes in patients with advanced HER2-positive gastric cancer compared to chemotherapy alone, making it a standard treatment regimen.[13] Additionally, trastuzumab combined with chemotherapy has been found to increase the pCR rate in resectable locally advanced HER2-positive gastric cancer.[14] Several breast cancer studies have indicated that HER2 expression may change from diagnosis to post-NAT, and this change is associated with prognosis.[15–17] The GERCOR study was the first to identify HER2 expression alterations between diagnostic and surgical resection specimens in gastric cancer patients receiving NAC. Moreover, these alterations were found to be associated with pathological response.[18] Among patients with advanced HER2-positive gastric cancer, a decrease in HER2 expression

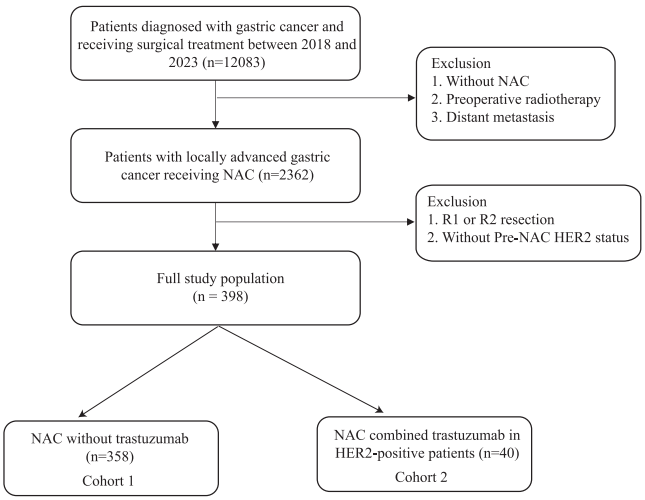

Figure 1. Flowchart of patients' selection. NAC, neoadjuvant chemotherapy

during trastuzumab combined with chemotherapy often indicates the development of trastuzumab resistance.[19,20] However, the biological mechanisms underlying alterations in HER2 expression after NAT and their prognostic significance in locally advanced gastric cancer remain unclear.

The primary objective of this study is to investigate the evolution of HER2 expression from diagnostic biopsy to post-NAT residual disease in consecutive patients with locally advanced gastric cancer. Additionally, we aim to further analyze the factors influencing HER2 expression alterations and explore the prognostic significance of these changes.

RESULTS

Patient characteristics

Figure 1 illustrates the process of patient screening and group allocation in this retrospective study. A total of 398 patients with locally advanced gastric cancer who received NAC followed by gastrectomy were included in this study. Of these, 358 patients did not receive trastuzumab during NAT and were classified as cohort 1 (Tables S1 and S2). Among cohort 1, 303 patients had residual disease after NAT and were included in the primary analysis (Table 1). The median age of patients in the primary analysis cohort was 59 years (range 29–80), with 71.0% being male (Table 1). At diagnosis, the HER2 status was primarily IHC 0 (60.7%), with only 6.3% classified as IHC 3+. Most patients did not receive targeted therapy during NAT, but nearly half received PD-1/PD-L1 inhibitors. Cohort 2 comprised 40 HER2-positive patients who received trastuzumab during NAT (Table S3). The median age in cohort 2 was 64 years (range 32–77), with most patients being male (85.0%). Additionally, 32 patients in cohort 2 had residual disease after NAT.

Response to neoadjuvant therapy

In the full study population, 15.8% (63/398) of patients achieved pCR. The pCR rate in cohort 1 was 15.4% (55/358), which was lower than the 20.0% (8/40) observed in cohort 2. The pCR rates varied according to HER2 status at diagnosis, with the highest rate observed in cohort 1 patients with HER2 IHC 2+ tumor (17.1%), while no pCR was observed in those with IHC 3+ tumor (Figure 2). In cohort 2, the addition of trastuzumab to NAT significantly increased pCR rates compared to patients with HER2 IHC 3+ tumor in cohort 1. TRG scores for all patients are presented in Table 2.

The use of PD-1/PD-L1 inhibitors during NAT significantly increased pCR rates, except in cohort 1 patients with HER2 IHC 1+ or 3+ tumors (Figure S1; Tables S4–S6). In cohort 2, the pCR rate was 27.3% for patients who received PD-1/PD-L1 inhibitors during NAT, whereas it was only 11.1% for those who did not (Table S7). However, this difference did not reach statistical significance, possibly due to the small sample size. Multivariate analysis in cohort 1 identified tumor size, PD-1/PD-L1 inhibitor, and Lauren classification as significant factors associated with pCR (Table S8). Diffuse (or mixed) Lauren type and tumors larger than 3 cm were unfavorable factors for achieving pCR, whereas receiving PD-1/PD-L1 inhibitors during NAT was associated with a higher pCR rate.

Alteration in HER2 expression

Among cohort 1 non-pCR patients, 129 (43.5%) experienced an alteration in HER2 expression from diagnosis to post-NAT, with 55.0% (71/129) showing decreased expression and 45.0% (58/129) showing increased expression (Table 3). In cohort 2, 16 patients (50%) with residual disease at surgery exhibited a decrease in HER2 expression from diagnosis to post-NAT. Detailed alterations in HER2 expression from diagnosis to post-NAT among patients with residual disease are presented in Figure 3. Alterations in HER2 expression were most frequently observed in patients with HER2 IHC 2+ tumors at diagnosis (Table 4). The rate of HER2 expression alteration in cohort 2 (50.0%, 16/32) was comparable to that of cohort 1 patients with HER2 IHC 3+ tumors (52.6%, 10/19).

Among cohort 1 non-pCR patients, the use of PD-1/PD-L1 inhibitors during NAT did not significantly affect the rate of HER2 expression change (Figure S2; Table S9). In cohort 2, patients with residual disease who received immunotherapy during NAT exhibited a slightly lower rate of HER2 expression alteration compared to those who did not receive immunotherapy (Figure S3; Table S10). Multivariate analysis of HER2 expression alteration among cohort 1 non-pCR patients identified HER2 status at diagnosis as a significant factor (Table S11). Patients with HER2 IHC 2+ tumors at diagnosis demonstrated an increased tendency for HER2 expression alterations after NAT.

Recurrence-free survival

In cohort 1, the median recurrence-free survival (RFS) for non-pCR patients was 33 months, while the median RFS for pCR patients was not reached (Figure 4A). Among cohort 1 non-pCR patients, the median RFS was 34 months for those with unchanged HER2 expression, 39 months for those with decreased expression, and 18 months for those with increased expression. Patients with decreased HER2 expression had the highest median RFS, but it was not significantly different from those with unchanged HER2 expression (Figure 4C). Patients with increased HER2 expression had the lowest median RFS, showing

Table 1. Demographics and disease characteristics of cohort 1 non-pCR patients

Characteristics	Total (*n* = 303)	IHC 0 (*n* = 184, 60.7%)	IHC 1 (*n* = 66, 21.8%)	IHC 2 (*n* = 34, 11.2%)	IHC 3 (*n* = 19, 6.3%)	P
Sex						
Male	215 (71.0%)	129 (70.1%)	46 (69.7%)	27 (79.4%)	13 (68.4%)	0.716
Female	88 (29.0%)	55 (29.9%)	20 (30.3%)	7 (20.6%)	6 (31.6%)	
Age						
≤60	168 (55.4%)	101 (54.9%)	40 (60.6%)	18 (52.9%)	9 (47.4%)	0.724
>60	135 (44.6%)	83 (45.1%)	26 (39.4%)	16 (47.1%)	10 (52.6%)	
Site						
EGJ	93 (30.7%)	55 (29.9%)	21 (31.8%)	12 (35.3%)	5 (26.3%)	0.782
Stomach body	89 (29.4%)	57 (31.0%)	21 (31.8%)	7 (20.6%)	4 (21.1%)	
Antrum	121 (39.9%)	72 (39.1%)	24 (36.4%)	15 (44.1%)	10 (52.6%)	
Size						
≤3	102 (33.7%)	59 (32.1%)	19 (28.8%)	15 (44.1%)	9 (47.4%)	0.219
>3	201 (66.3%)	125 (67.9%)	47 (71.2%)	19 (55.9%)	10 (52.6%)	
Lauren type						
Intestine	122 (40.3%)	66 (35.9%)	24 (36.4%)	19 (55.9%)	13 (68.4%)	0.048
Diffused	112 (37.0%)	77 (41.8%)	24 (36.4%)	8 (23.5%)	3 (15.8%)	
Mixed	69 (22.7%)	41 (22.3%)	18 (27.2%)	7 (20.6%)	3 (15.8%)	
Differentiation						
Poor	216 (71.3%)	142 (77.2%)	47 (71.2%)	21 (61.8%)	6 (31.6%)	<0.001
Moderate	87 (28.7%)	42 (22.8%)	19 (28.8%)	13 (38.2%)	13 (68.4%)	
Chemotherapy regimen						
FLOT/FOLFOX	27 (8.9%)	17 (9.2%)	4 (6.1%)	5 (14.7%)	1 (5.3%)	0.527
SOX/XELOX	276 (91.1%)	167 (90.8%)	62 (93.9%)	29 (85.3%)	18 (94.7%)	
Chemotherapy cycle						
≤3	119 (33.2%)	69 (31.2%)	23 (29.9%)	18 (43.9%)	9 (47.4%)	0.243
>3	239 (66.8%)	152 (68.8%)	54 (70.1%)	23 (56.1%)	10 (52.6%)	
PD-1/PD-L1 inhibitor						
Without	157 (51.8%)	102 (55.4%)	30 (45.5%)	15 (44.1%)	10 (52.6%)	0.416
With	146 (48.2%)	82 (44.6%)	36 (54.5%)	19 (55.9%)	9 (47.4%)	
Targeted therapy						
Without	264 (87.1%)	160 (87.0%)	60 (90.9%)	28 (82.4%)	16 (84.2%)	0.574
VEGF-inhibitor	39 (12.9%)	24 (13.0%)	6 (9.1%)	6 (17.6%)	3 (15.8%)	
Operation						
Partial gastrectomy	165 (54.5%)	95 (51.6%)	28 (42.4%)	27 (79.4%)	15 (78.9%)	0.001
Total gastrectomy	138 (45.5%)	89 (48.4%)	38 (57.6%)	7 (20.6%)	4 (21.1%)	
ypT						
T1	38 (12.5%)	20 (10.9%)	9 (13.6%)	7 (20.7%)	2 (10.5%)	<0.001
T2	37 (12.2%)	20 (10.9%)	9 (13.6%)	3 (8.8%)	5 (26.3%)	
T3	102 (33.7%)	56 (30.4%)	23 (34.9%)	18 (52.9%)	5 (26.3%)	
T4	126 (41.6%)	88 (47.8%)	25 (37.9%)	6 (17.6%)	7 (36.9%)	
ypN						
N0	108 (35.6%)	69 (37.5%)	20 (30.3%)	11 (32.4%)	8 (42.1%)	<0.001
N1	66 (21.8%)	37 (20.1%)	16 (24.3%)	11 (32.4%)	2 (10.5%)	
N2	55 (18.2%)	27 (14.7%)	15 (22.7%)	5 (14.6%)	8 (42.1%)	
N3	74 (24.4%)	51 (27.7%)	15 (22.7%)	7 (20.6%)	1 (5.3%)	

EGJ, esophagogastric junction.

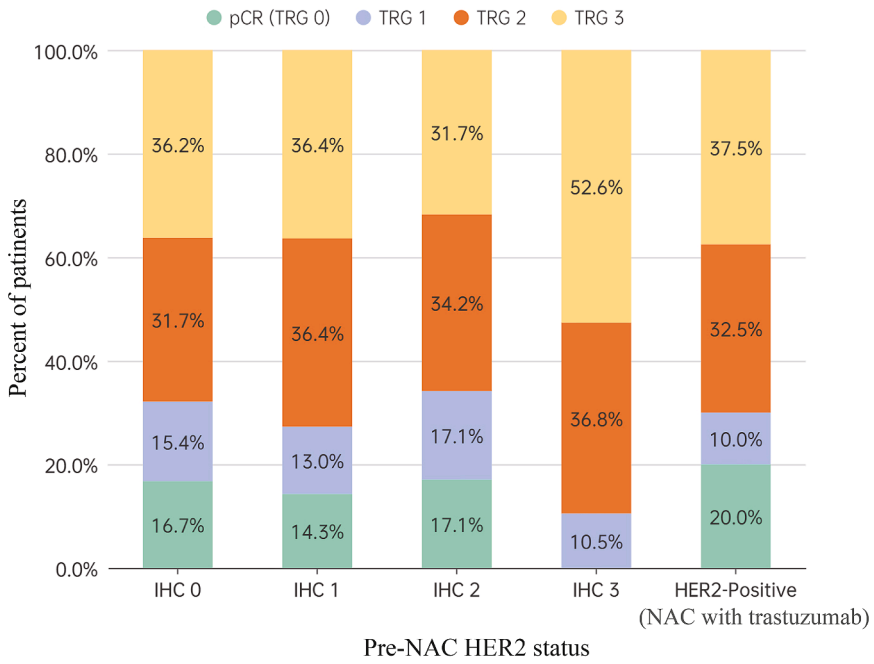

Figure 2. Post-neoadjuvant therapy response status by HER2 status at primary diagnosis among full study population

significant differences compared to the other groups (Figures 4B and 4D). In cohort 2, the median RFS for pCR and non-pCR patients was 38 months and 30 months, respectively (Figure S4A). For cohort 2 non-pCR patients, the median RFS was 38 months for those with decreased HER2 expression and 29 months for those with unchanged HER2 expression, but the difference was not statistically significant (Figure S4B). The post-recurrence treatment regimens for patients in cohort 1 and cohort 2 are separately summarized in Tables S12 and S13.

The median RFS was 36 months for patients in cohort 1 who received PD-1/PD-L1 inhibitors during NAT and 34 months for those who did not; however, this difference was not statistically significant (Figure S5A). Similarly, in cohort 2, the median RFS was 34 months for patients who received PD-1/PD-L1 inhibitors during NAT and 32 months for those who did not, with no statistically significant difference observed (Figure S5B).

Overall survival

In cohort 1, the median OS for non-pCR patients was 45 months, while the median OS for pCR patients was not reached (Figure 5A). For cohort 1 non-pCR patients, the median OS was 45 months for those with unchanged HER2 expression, 53 months for those with decreased expression, and 27 months for those with increased expression (Figures 5B–5D). There was no significant difference in median OS between patients with decreased and unchanged HER2 expression. In cohort 2, the median OS was 51 months for pCR patients and 42 months for non-pCR patients (Figure S6A). For cohort 2 non-pCR patients, the median OS was 45 months for those with decreased HER2 expression and 40 months for those with unchanged HER2 expression, but the difference was not statistically significant (Figure S6B).

The median OS was 50 months for patients who received PD-1/PD-L1 inhibitors during NAT in cohort 1, compared to 45 months for those who did not (Figure S7A); however, this difference was not statistically significant. Similarly, in cohort 2, patients who received PD-1/PD-L1 inhibitors had a median OS of 45 months, while those who did not had a median OS of 42 months (Figure S7B), with no statistically significant difference observed.

DISCUSSION

In this study, we retrospectively collected data on patients with locally advanced gastric cancer who received NAT followed by surgery. We assessed changes in HER2 expression in consecutive patients from diagnosis to post-NAT. Among patients with residual disease after NAT, the rate of HER2 expression change was 43.5% in cohort 1 and 50% in cohort 2. In cohort 1, the proportion of patients with decreased HER2 expression (23.4%) was higher than that of patients with increased expression (19.1%). Patients with HER2 IHC 2+ at diagnosis demonstrated the highest rate of HER2 expression change after NAT. Furthermore, multivariate analysis revealed that HER2 status at diagnosis influenced subsequent changes in HER2 expression.

Trastuzumab, a targeted agent against HER2 overexpression, was initially approved for breast cancer treatment.[21] Since the ToGA study, it has become an integral part of the standard

Table 2. Post-neoadjuvant therapy (NAT) response status among patients in full study population

	HER2 status at primary diagnosis						
Post-NAT response	IHC 0	IHC 1+	IHC 2+	IHC 3+	Cohort 2	Total	*p* value
pCR (TRG 0)	37 (16.7%)	11 (14.2%)	7 (17.1%)	0	8 (20.0%)	63	
TRG 1	34 (15.4%)	10 (13.0%)	7 (17.1%)	2 (10.5%)	4 (10.0%)	57	
TRG 2	70 (31.7%)	28 (36.4%)	14 (34.1%)	7 (36.8%)	13 (32.5%)	132	
TRG 3	80 (36.2%)	28 (36.4%)	13 (31.7%)	10 (52.7%)	15 (37.5%)	146	
Total	221	77	41	19	40	398	<0.001

Table 3. Summary of alteration in HER2 expression in full study population with residual disease at surgery

	Alteration in HER2				
Patients with residual disease	Reduced expression	Increased expression	Unchanged	Total	*p* value
Cohort 1	71 (23.4%)	58 (19.1%)	174 (57.5%)	303	
Cohort 2	16 (50.0%)	0	16 (50.0%)	32	
Total	87 (26.0%)	58 (17.3%)	190 (56.7%)	335	<0.001

treatment for advanced HER2-positive gastric cancer.[13] Its role in neoadjuvant therapy for HER2-positive locally advanced gastric cancer is actively being explored in clinical studies.[22,23] A small-sample retrospective study found that the addition of trastuzumab to NAT did not significantly improve survival in HER2-positive gastric cancer, but its inclusion in postoperative adjuvant therapy was associated with improved survival outcomes.[14] Some studies have reported changes in HER2 expression in advanced HER2-positive gastric cancer following trastuzumab administration, with decreased HER2 expression often indicating treatment failure.[19,24] HER2 expression changes in tumor cells are one of the mechanisms of tumor resistance to trastuzumab.[25,26] In our study, all HER2-positive gastric cancer patients in cohort 2 received trastuzumab as part of their neoadjuvant therapy, and 50% of patients with residual disease showed a decrease in HER2 expression. The use of trastuzumab significantly increased the pCR rate in HER2-positive gastric cancer patients. However, since there were only 40 patients in cohort 2, the small sample size may not adequately reflect the trends in HER2 expression changes. When comparing with cohort 1, the sample size imbalance may have contributed to the observed statistical significance. Given this limitation, future studies should include a larger cohort of HER2-positive locally advanced gastric cancer patients to further validate these findings.

Immunotherapy is recommended for HER2-positive advanced gastric cancer with a PD-L1 combined positive score (CPS) $\geq$1, and for HER2-negative advanced gastric cancer with a PD-L1 CPS $\geq$5.[27,28] Recently, its use in the NAT of locally advanced gastric cancer has increased.[29–31] The KEYNOTE-585 study demonstrated that perioperative immunotherapy could improve the pCR rate, though it did not lead to a significant improvement in patient survival.[7] In our study, immunotherapy increased the pCR rate in both cohort 1 and cohort 2. However, among patients with residual disease, it did not significantly influence changes in HER2 expression. PD-1/PD-L1 inhibitors enhance T cell-mediated tumor killing by blocking the interaction between PD-L1 on tumor cells and PD-1 on T cells. Since these inhibitors do not directly affect HER2-related signaling pathways, this may explain why they increase pCR rates without significantly altering HER2 expression.

Changes in HER2 expression after NAT have been associated with prognosis in breast cancer, where patients with decreased HER2 expression tend to have better outcomes.[15] In our study, patients who achieved pCR had better survival outcomes compared to non-pCR patients. Among non-pCR patients in cohort 1, those with decreased HER2 expression had the best prognosis, while those with increased expression had the worst prognosis. However, survival differences between patients with decreased and unchanged HER2 expression were not statistically significant. In cohort 2 non-pCR patients, the prognosis was similar between those with decreased and unchanged HER2 expression, which may be attributed to the limited sample size. Our study suggests that a decrease in HER2 expression after NAT is a favorable prognostic factor for locally advanced gastric cancer, although further research is needed to confirm this finding. This decrease may be associated with increased sensitivity of tumor

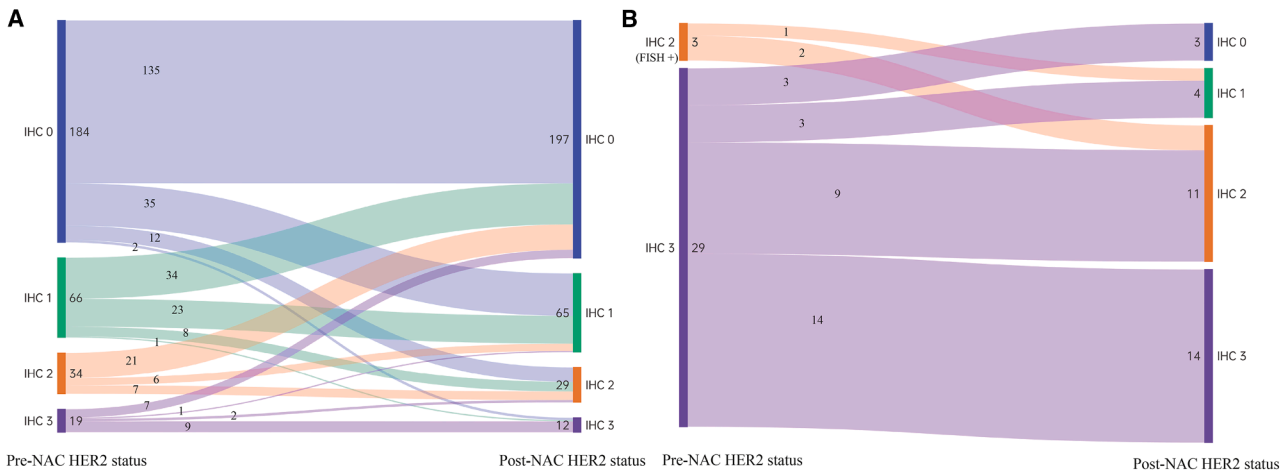

Figure 3. HER2 expression evolution among patients with residual disease at surgery
(A) Cohort 1; (B) cohort 2.

Table 4. Alteration in HER2 expression by HER2 status at primary diagnosis among the full study population with residual disease at surgery

	HER2 status at primary diagnosis						
Alteration in HER2	IHC 0	IHC 1+	IHC 2+	IHC 3+	Cohort 2	Total	*p* value
Down-regulated	0	34 (51.5%)	27 (79.4%)	10 (52.6%)	16 (50.0%)	87	
Up-regulated	49 (26.6%)	9 (13.7%)	0	0	0	58	
Unchanged	135 (73.4%)	23 (34.8%)	7 (20.6%)	9 (47.4%)	16 (50.0%)	190	
Total	184	66	34	19	32	335	<0.001

cells to NAT.[18] Chemotherapy, immunotherapy, and targeted therapy in NAT are all systemic treatments. The heightened sensitivity of tumor cells to these therapies may facilitate the elimination of undetected micrometastases, which could contribute to a lower risk of postoperative recurrence and better survival outcomes. For patients with decreased HER2 expression, if postoperative recurrence requires chemotherapy, the initial NAC regimen can be prioritized. Additionally, HER2 status should be re-evaluated in recurrent lesions. This reassessment not only helps determine the need for HER2-targeted therapy but also allows for a comparison between HER2 expression in recurrent and post-NAT lesions. Currently, studies on HER2 expression changes between recurrent and post-NAT lesions are limited, and further research is needed to elucidate the underlying mechanisms and prognostic significance.

In our study, most patients with postoperative recurrence underwent multimodal treatment, including chemotherapy,

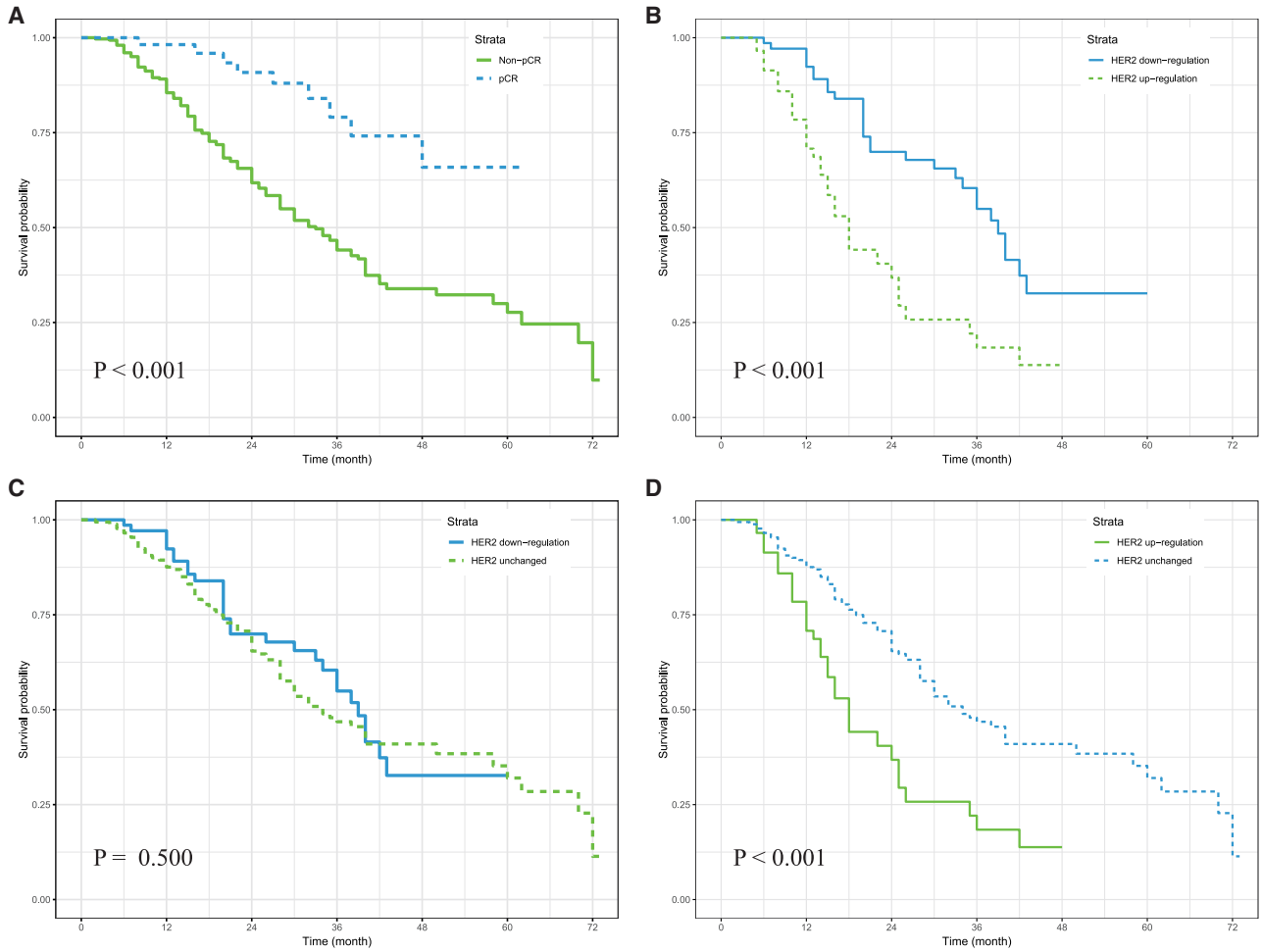

Figure 4. Recurrence-free survival

(A) By pathological complete response (pCR) status in cohort 1.

(B–D) By change in HER2 expression among cohort 1 non-pCR patients.

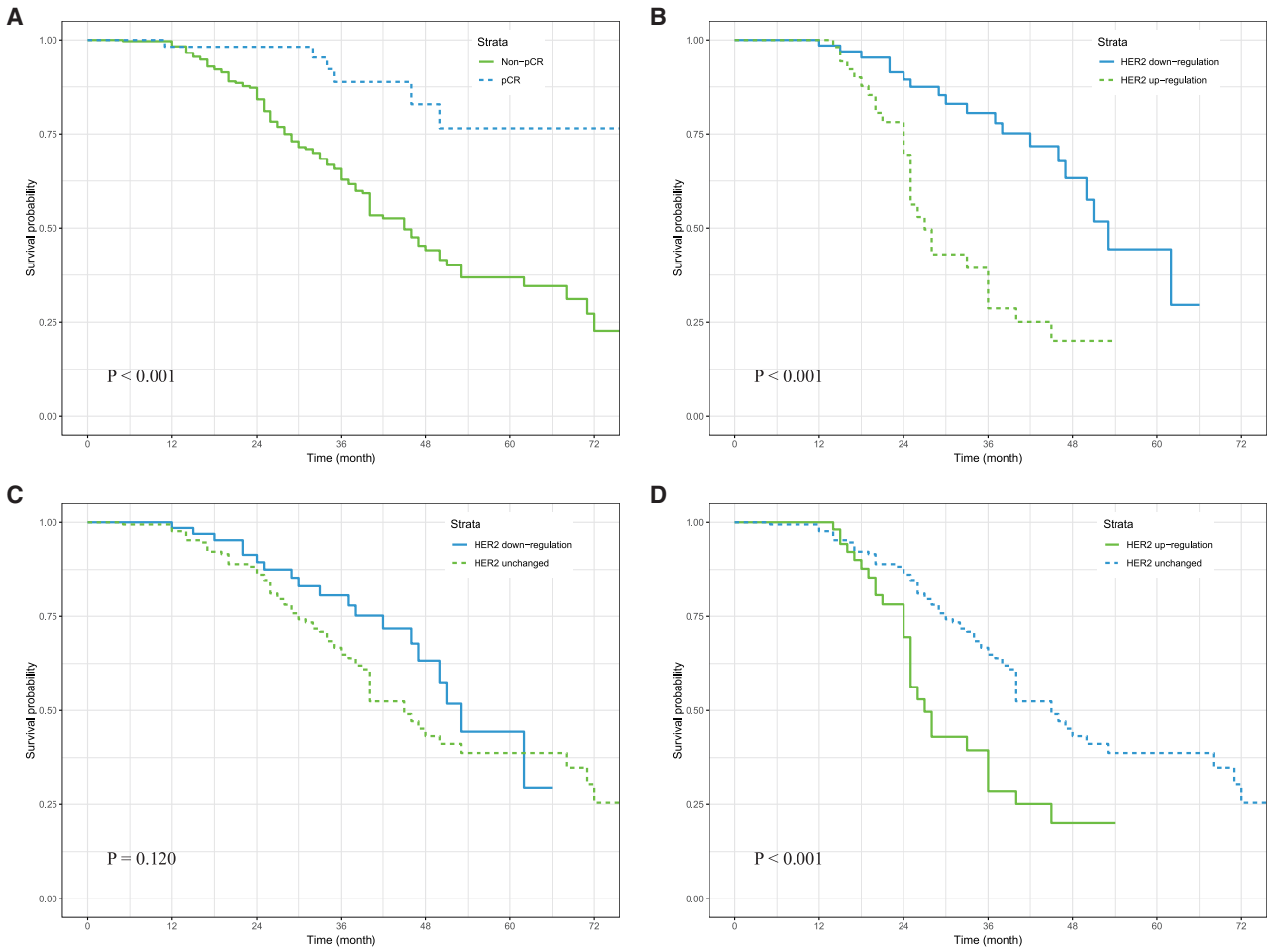

Figure 5. Overall survival

(A) By pathological complete response (pCR) status in cohort 1.

(B–D) By change in HER2 expression among cohort 1 non-pCR patients.

immunotherapy, targeted therapy, and radiotherapy. A small subset of patients also received surgical treatment. The decision to administer HER2-targeted therapy was primarily based on HER2 IHC (in situ hybridization [ISH]) results from the biopsy or surgical specimen of the recurrent lesion. However, for a small number of patients who were not suitable for biopsy, HER2-targeted therapy was administered based on the HER2 assessment of the gastric tumor after NAT. The biological characteristics of recurrent lesions may differ significantly from those of post-NAT lesions. Therefore, when postoperative recurrence occurs, we recommend performing a biopsy of the recurrent lesion to determine its histological type and HER2 status. This reassessment can provide valuable guidance for immunotherapy and HER2-targeted therapy.

Tumor responsiveness to NAT can influence changes in HER2 expression.[32] HER2-positive gastric cancer usually exhibits greater sensitivity to chemotherapy.[33] In our study, we observed a higher rate of HER2 downregulation compared to upregulation. Notably, none of the HER2-positive gastric cancer patients in cohort 1 achieved pCR, while the pCR rate reached 20% in cohort 2, suggesting that trastuzumab can significantly enhance the pathological response. Tumor heterogeneity may contribute to discrepancies in HER2 status, possibly due to differences in sampling sites between diagnostic and postoperative specimens.[34,35] Given the potential for HER2 expression changes, reassessing HER2 status in postoperative specimens is crucial.

This multicenter retrospective study revealed that HER2 expression alterations occurred in over 40% of locally advanced gastric cancer patients who underwent NAT and did not achieve pCR. Clinicians should consider HER2 as a crucial biomarker when managing patients with locally advanced gastric cancer. However, this study has certain limitations. Being retrospective, it is subject to selection bias. Additionally, the short follow-up period resulted in neither the median RFS nor the median OS being reached among pCR patients in cohort 1. To address these limitations, future basic research and prospective clinical studies are needed to elucidate the biological mechanisms of HER2 expression changes in gastric cancer and their prognostic significance.

Conclusion

This study found that HER2 expression alterations occurred in over 40% of locally advanced gastric cancer patients who underwent NAT and did not achieve pCR, with a higher incidence of decreased expression (26.0%) compared to increased expression (17.3%). Additionally, we found that decreased HER2 expression was associated with better prognosis; however, this requires further experimental verification. Both trastuzumab and PD-1/PD-L1 inhibitors improved the pCR rates, but neither significantly impacted the rate of HER2 expression alterations among non-pCR patients.

Limitations of the study

Several limitations should be acknowledged in this study. First, although patients were selected from three major medical centers in China, all cases were identified retrospectively. This may introduce a risk of selection bias, despite the application of strict inclusion criteria. Second, the follow-up period was relatively short. As a result, neither the median RFS nor the median OS was reached in the pCR group of cohort 1. Third, cohort 2 included only 40 patients with HER2-positive locally advanced gastric cancer. This limited sample size may reduce the statistical power and may not fully reflect the heterogeneity of HER2 expression changes after neoadjuvant therapy. Finally, although this study revealed dynamic changes in HER2 status after neoadjuvant therapy, the underlying molecular mechanisms remain unclear. Future mechanistic studies are needed to investigate the biological basis of these changes.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Weihua Gong (weihuagong@zju.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Date: raw data have been deposited at Mendeley data and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- Code: this article does not report the original code.
- Any additional information required to reanalyze the data reported in this article is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: J.H., W.G., Y.L., and Y.Z.; data collection and check: B.L., N.Z., Y.D., X.S., Y.L., H.W., C.T., J.D., R.Z., and X.W.; methodology: J.H., M.L., and Y.D.; formal analysis and investigation: Y.D. and C.H.; writing – original draft preparation: J.H., H.L., Y.D., and C.H.; Writing – review and editing: Y. Z., Y.L., W.G., D.S., and D.C.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data	This paper; Mendeley Data	https://data.mendeley.com/preview/nbm799jkbc
Software and algorithms		
R studio version 4.4.3	R software	https://www.rstudio.com/tags/rstudio/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Ethics approval

This study was approved by the Ethics Committee of the Second Affiliated Hospital Zhejiang University School of Medicine (No.2024-1147).

Study population

We retrospectively collected data on patients with locally advanced gastric cancer who received NAC followed by gastrectomy between 2018 and 2023 at three medical centers. All human participants were of Asian race and Chinese ethnicity. The study population was selected based on strict inclusion and exclusion criteria. The inclusion criteria for patients were: (1) confirmed adenocarcinoma; (2) HER2 expression assessed at diagnostic biopsy; (3) received NAC; (4) underwent gastrectomy; (5) HER2 expression assessed in postoperative specimens; (6) proficient mismatch repair (pMMR) status. Exclusion criteria were: (1) received radiotherapy before surgery; (2) R1 or R2 resection; (3) presence of distant metastasis; (4) presence of other synchronous tumors.

METHOD DETAILS

Grouping

In total, 398 patients with locally advanced gastric cancer were included as the full study population, comprising 238 patients from Cancer Hospital Chinese Academy of Medical Sciences, 157 from Tianjin Medical University Cancer Institute and Hospital, and 3 from the Second Affiliated Hospital Zhejiang University School of Medicine. Among these patients, 358 did not receive trastuzumab in the NAT regimen (Cohort 1), while 40 HER2-positive gastric cancer patients received trastuzumab in the NAT regimen (Cohort 2). Moreover, 13 patients who received NAC with PD-1/PD-L1 inhibitor were part of the DRAGON IV study (ClinicalTrials.gov ID: NCT04208347).

HER2 status assessment

Prior to neoadjuvant therapy, tumor tissue is obtained through biopsy for immunohistochemical (IHC) testing. At three medical centers, at least three biopsy samples are collected for HER2 assessment, covering both the tumor core and invasive margins, based on tumor size, location, and morphology. After gastrectomy, the tumor undergoes whole-section IHC evaluation. Two independent pathologists assess the HER2 IHC intensity. In case of disagreement, a third senior pathologist is consulted to resolve the discrepancy and determine the IHC result. Tumors with an IHC staining intensity of 2+ are further analyzed using fluorescence *in situ* hybridization (FISH) to confirm HER2 gene amplification. HER2 positivity is defined as IHC 3+ or IHC 2+ with FISH indicating gene amplification.

QUANTIFICATION AND STATISTICAL ANALYSIS

Clinical and pathological information of patients included gender, age, tumor location, tumor size, Lauren type, differentiation, chemotherapy regimen, number of chemotherapy cycles, immunotherapy, targeted therapy, surgery, HER2 status at diagnosis, tumor regression grade (TRG), ypT, and ypN. HER2 positivity is defined as immunohistochemistry (IHC) 3+ or IHC 2+ with *in situ* hybridization (ISH) indicating gene amplification. HER2 expression alteration refers to the change in HER2 expression from diagnostic biopsy to residual disease after NAT in consecutive patients. Recurrence-free survival (RFS) is defined as the interval from gastrectomy to tumor recurrence or death, while OS is defined as the interval from gastric cancer diagnosis to death. The median follow-up time for all patients was 41 months, with the follow-up cutoff date set at November 1, 2024.

Categorical variables were compared using the χ^2 (Fisher's exact) tests. The Kaplan-Meier (KM) method and log-rank test were used to compare OS and RFS between groups. The full study population was used for pCR analysis, while patients with residual disease after NAT were analyzed for changes in HER2 expression. Logistic regression analysis was performed to identify factors

influencing pCR and HER2 expression alteration. Variables with statistical significance in univariate analysis were included in the multivariate analysis. All statistical analyses were performed using R version 4.4.0, with $p < 0.05$ considered statistically significant.

ADDITIONAL RESOURCES

13 patients who received NAC with PD-1/PD-L1 inhibitor were part of the DRAGON IV study (ClinicalTrials.gov ID: NCT04208347). Further information is available at: https://clinicaltrials.gov/.