

Immune-Depleted Features of HER2-Expressing Gastric Cancer and Immune Remodeling After Trastuzumab-Containing Therapy

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Abstract

Purpose

Immune checkpoint inhibitors (ICIs) have become integral to the treatment of gastric cancer, and pembrolizumab combined with trastuzumab and chemotherapy has improved outcomes in HER2-positive advanced disease. However, the baseline immune phenotype of HER2-expressing gastric cancer and the changes induced by anti-HER2 therapy remain incompletely defined. We investigated the local immune microenvironment associated with HER2 expression and explored immune changes after trastuzumab-containing preoperative therapy.

Methods

Three complementary analyses were performed. First, HER2 expression, CD8 + T-cell infiltration, and PD-L1 combined positive score (CPS) were assessed by immunohistochemistry in 64 gastrectomy specimens without preoperative therapy. Second, The Cancer Genome Atlas gastric cancer dataset was used to examine ERBB2 amplification in relation to somatic mutation count, cytolytic activity (CYT) score, and CIBERSORT-estimated immune-cell fractions. Third, paired pretreatment biopsy and post-treatment resection specimens from six patients with HER2-positive gastric cancer who received trastuzumab-containing preoperative therapy were analyzed for CD8, PD-L1, PD-L2, SIRP α , and CD80.

Results

The IHC 2+/3 + group showed significantly lower CD8 + T-cell infiltration and lower PD-L1 CPS than the IHC 0/1 + group ($P = 0.04$ and $P = 0.03$, respectively). In TCGA, ERBB2-amplified tumors had lower somatic mutation counts and CYT scores (both $P < 0.0001$), together with lower estimated fractions of several immune-cell populations, including CD8 + T cells. In the six paired cases, CD8, PD-L1, PD-L2, and SIRP α increased significantly after therapy, whereas CD80 did not significantly change.

Conclusion

Higher HER2 expression and ERBB2 amplification were associated with an immune-depleted phenotype in gastric cancer. Conversely, trastuzumab-containing therapy was associated with increased CD8 + T-cell infiltration and checkpoint-related molecule expression, suggesting treatment-associated remodeling toward a more inflamed tumor microenvironment. These findings provide a biological framework for combining HER2-directed therapy with PD-1/PD-L1 blockade.

Introduction

Human epidermal growth factor receptor 2 (HER2; ERBB2) is a receptor tyrosine kinase within the ErbB family. HER2 overexpression or ERBB2 amplification promotes constitutive signaling through the RAS-RAF-MEK-ERK and PI3K-AKT pathways, supporting tumor-cell proliferation and survival [1, 2]. In gastric and gastroesophageal junction (GEJ) adenocarcinoma, HER2 is a clinically established biomarker and therapeutic target. Approximately 15–25% of tumors show HER2 overexpression or amplification, with enrichment in intestinal-type and differentiated tumors [3–5].

The phase III ToGA trial established trastuzumab plus chemotherapy as a standard first-line treatment for HER2-positive advanced gastric/GEJ cancer [5]. More recently, trastuzumab deruxtecan has expanded HER2-directed treatment options in previously treated disease [6]. At the same time, immune checkpoint inhibitors have transformed systemic therapy for gastric cancer. In HER2-positive disease, KEYNOTE-811 demonstrated that the addition of pembrolizumab to trastuzumab and chemotherapy improves antitumor activity and progression-free survival, supporting a therapeutic interaction between HER2-directed treatment and PD-1 blockade [7].

This interaction is biologically plausible because trastuzumab is not solely a signaling inhibitor. As an IgG1 monoclonal antibody, it can engage Fcγ receptor-expressing immune cells and induce antibody-dependent cellular cytotoxicity (ADCC), thereby promoting tumor-cell killing and antigen release. Experimental and translational studies have shown that anti-HER2 antibodies can engage innate and adaptive antitumor immunity, while anticancer therapy can also induce adaptive immune-resistance programs, including increased PD-L1 expression [8–10]. Thus, anti-HER2 therapy may alter the tumor microenvironment (TME) in ways that are relevant to ICI sensitivity.

Nevertheless, two questions remain clinically important. First, the baseline immune phenotype associated with higher HER2 expression in gastric cancer has not been fully characterized across pathologic and genomic levels. Second, the direction and magnitude of immune changes occurring after trastuzumab-containing treatment remain incompletely understood. The present study addressed these questions through three complementary analyses: an institutional immunohistochemical cohort, an in silico analysis of TCGA gastric cancers, and paired tissue analysis before and after trastuzumab-containing preoperative therapy. We hypothesized that higher HER2 expression would be associated with an immune-depleted baseline phenotype, whereas trastuzumab-containing treatment would be associated with local immune activation and induction of checkpoint-related molecules.

Materials and Methods

Study design

This study comprised three complementary components. First, we evaluated HER2 expression, CD8 + T-cell infiltration, and PD-L1 expression in resected gastric cancers from a single institutional cohort. Second, we analyzed TCGA gastric cancer data to assess associations between ERBB2 amplification and genomic/immune parameters, including somatic mutation count, CYT score, and CIBERSORT-estimated immune-cell composition. Third, we performed paired immunohistochemical analyses of

pretreatment biopsy and post-treatment resection specimens from patients with HER2-positive gastric cancer who received preoperative trastuzumab-containing therapy.

Institutional gastrectomy cohort

Consecutive patients who underwent gastrectomy for primary gastric cancer at Kyushu University Hospital in 2014–2015 were screened. Formalin-fixed paraffin-embedded (FFPE) tissue and clinicopathologic information were required. T1a tumors, cases with a history of preoperative treatment, specimens with insufficient tissue or inadequate quality, and cases with missing key variables were excluded. Sixty-four patients were included in the final IHC analysis. Exclusion of pretreated tumors was intended to characterize the treatment-naïve immune microenvironment associated with HER2 expression.

Paired pre- and post-treatment cohort

Six patients with HER2-positive gastric cancer underwent gastrectomy at Kyushu University after preoperative trastuzumab-containing therapy between 2011 and 2020 and had both pretreatment biopsy tissue and matched post-treatment resection tissue available for analysis. These paired samples were used to quantify treatment-associated changes in CD8 and immune checkpoint-related molecules.

Immunohistochemistry

FFPE sections (4 µm) were deparaffinized and rehydrated, followed by blockade of endogenous peroxidase activity. Heat-induced epitope retrieval was performed using citrate buffer at pH 6.0 (98°C for 40 min) or Tris-EDTA buffer at pH 8.0 (98°C for 20 min), according to the target antigen. HER2 was stained using an anti-human c-erbB-2 oncoprotein rabbit monoclonal antibody (Dako; 1:200). CD8 was stained using clone C8/144B (Dako; mouse monoclonal; 1:100). PD-L1 was assessed using clone 28 – 8. PD-L2, SIRPα, and CD80 were additionally evaluated in the paired pre-/post-treatment specimens. Detection was performed using an HRP-DAB polymer method followed by hematoxylin counterstaining.

Scoring of HER2, CD8, and PD-L1

HER2 membrane staining was scored as 0, 1+, 2+, or 3+ according to established gastric cancer scoring criteria [11, 12]. For the exploratory institutional analysis, tumors were grouped as IHC 0/1+ versus IHC 2+/3+. Importantly, this grouping was used to evaluate HER2 protein-expression level rather than to define clinical HER2 positivity; in routine clinical practice, IHC 2+ tumors require confirmation of ERBB2 amplification by in situ hybridization [11, 12]. Accordingly, throughout this manuscript the IHC 2+/3+ category is referred to as the higher-HER2-expression group when clinical HER2 positivity has not been independently confirmed.

CD8+ cells were counted in five non-overlapping high-power fields (×400) at representative hotspots along the invasive front, and the mean number of positive cells per high-power field (cells/HPF) was used as the case-level value. PD-L1 was evaluated using the combined positive score (CPS), calculated

as the number of PD-L1-positive tumor cells, lymphocytes, and macrophages divided by the total number of viable tumor cells and multiplied by 100. For the paired pre-/post-treatment cohort, changes in each marker were assessed within the same patient.

TCGA in silico analysis

RNA-sequencing, whole-exome sequencing, and copy-number data were obtained from the TCGA gastric adenocarcinoma dataset [13]. CIBERSORT was used to estimate relative immune-cell fractions in 415 tumors [14]. Somatic mutation counts were available for 393 tumors, and CYT scores were available for 413 tumors. The CYT score was calculated as the geometric mean of GZMA and PRF1 expression levels, i.e., $\text{CYT} = \sqrt{(\text{GZMA} \times \text{PRF1})}$, as an index of intratumoral cytolytic immune activity, consistent with the metric originally described by Rooney et al. [15]. ERBB2 amplification was defined using the copy-number threshold applied in the original analysis (copy number ≥ 2.5 in the results dataset). Because mutation burden was analyzed as the total number of somatic mutations rather than mutations per megabase, we refer to this variable as somatic mutation count rather than tumor mutational burden (TMB) in the main text.

Endpoints and statistical analysis

The principal endpoints were: (1) differences in CD8 + T-cell infiltration and PD-L1 CPS between the IHC 0/1 + and IHC 2+/3 + groups; (2) associations of ERBB2 amplification with somatic mutation count, CYT score, and CIBERSORT-estimated immune-cell fractions; and (3) within-patient changes in CD8, PD-L1, PD-L2, SIRP α , and CD80 after trastuzumab-containing preoperative therapy. Continuous variables were compared using Student's t-test for approximately normally distributed data and the Mann–Whitney U test for non-normally distributed data. Paired data were analyzed using a paired t-test or Wilcoxon signed-rank test, as appropriate. Analyses were performed using R version 4.1 and Python. Two-sided P values < 0.05 were considered statistically significant.

Ethics and data availability

The institutional study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Kyushu University Hospital. Informed consent was obtained or waived in accordance with the committee-approved protocol. TCGA data were obtained from a publicly available repository.

Results

Higher HER2 expression was associated with reduced CD8+ T-cell infiltration and lower PD-L1 CPS

Among the 64 patients in the institutional gastrectomy cohort, HER2, CD8, and PD-L1 were evaluated by IHC. Clinicopathological characteristics according to HER2 IHC category are summarized in Table 1; no significant differences were observed for the variables shown. CD8+ T cells were quantified at the invasive front, and PD-L1 expression was quantified using CPS. The IHC 2+/3+ group had significantly

fewer infiltrating CD8+ T cells than the IHC 0/1+ group (P=0.04; Fig. 1A). PD-L1 CPS was also significantly lower in the IHC 2+/3+ group (P=0.03; Fig. 1B). These findings indicate that higher HER2 protein expression was associated with reduced cytotoxic T-cell infiltration and lower checkpoint-ligand expression in treatment-naïve resected gastric cancers. Representative HER2, CD8, and PD-L1 staining patterns are shown in Supplementary Fig. 1.

Table 1. Clinicopathological characteristics according to HER2 IHC category in the institutional cohort

Characteristic	IHC 0/1+ (n=46)	IHC 2+/3+ (n=18)	P value
Sex			0.83
Male	32 (70.0)	13 (72.2)	
Female	14 (30.0)	5 (27.8)	
Age, median, years	72.0	72.5	0.93
Surgical procedure			0.11
Distal gastrectomy	28 (60.9)	13 (72.2)	
Proximal gastrectomy	0 (0)	1 (5.6)	
Total gastrectomy	18 (39.1)	4 (22.2)	
Pathological T category			0.10
pT1b	8 (17.4)	6 (33.3)	
pT2	15 (32.6)	2 (11.1)	
pT3	15 (32.6)	8 (44.4)	
pT4	8 (17.4)	2 (11.1)	
Pathological N category			0.78
pN0	24 (52.2)	11 (61.1)	
pN1	7 (15.2)	3 (16.6)	
pN2	9 (19.6)	2 (11.1)	
pN3	6 (13.0)	2 (11.1)	
Pathological stage			0.95
I	18 (39.1)	8 (44.4)	
II	15 (32.6)	6 (33.3)	
III	11 (23.9)	4 (22.2)	
IV	2 (4.4)	0 (0)	

Values are n (%) unless otherwise indicated. The IHC 2+/3+ category denotes the higher-HER2-expression group used for this exploratory analysis and does not by itself establish clinical HER2 positivity for IHC 2+ tumors.

ERBB2-amplified tumors showed lower mutation counts, lower cytolytic activity, and reduced immune-cell fractions in TCGA

In TCGA, ERBB2-amplified tumors had significantly lower somatic mutation counts than non-amplified tumors ($P<0.0001$; $n=393$; amplified, $n=70$; non-amplified, $n=323$). CYT scores were also significantly lower in ERBB2-amplified tumors ($P<0.0001$; $n=413$; amplified, $n=75$; non-amplified, $n=338$) (Fig. 2A; Table 2). CIBERSORT analysis further demonstrated lower estimated fractions of several immune-cell populations in ERBB2-amplified tumors, including CD8+ T cells, M2 macrophages, resting mast cells, memory B cells, and activated CD4+ memory T cells (Fig. 2B). Taken together, these genomic and transcriptomic observations were directionally consistent with the institutional IHC findings and support an immune-depleted phenotype associated with ERBB2 amplification.

Table 2. Somatic mutation count and cytolytic activity according to ERBB2 amplification status in TCGA gastric cancer

Variable	ERBB2 amplified	Non-amplified	P value
Somatic mutation count	199 (43-1015) (n=70)	597 (32-8811) (n=323)	<0.0001
CYT score	6.2 (3.5-8.6) (n=75)	7.6 (3.7-10.9) (n=338)	<0.0001

Values are median (range). P values were calculated using the Mann–Whitney U test. CYT, cytolytic activity; TCGA, The Cancer Genome Atlas.

Immune-related markers increased after trastuzumab-containing preoperative therapy

Six patients with HER2-positive gastric cancer had paired pretreatment biopsy and post-treatment resection specimens available. After trastuzumab-containing therapy, PD-L1 CPS increased from a median of 9.0 to 17.2, PD-L2-positive cells increased from 7.3 to 12.0, SIRPα-positive cells increased from 1.9 to 4.4, and CD8+ cells increased from 8.5 to 16.6. These changes were statistically significant in the source analysis (approximately $P=0.02$ - 0.03). By contrast, CD80-positive cells changed only minimally (median 5.8 to 6.0) and were not significantly different ($P=0.78$) (Fig. 3). Thus, the post-treatment specimens showed greater cytotoxic T-cell infiltration together with increased expression of multiple immune checkpoint-related molecules.

Taken together, these findings indicate that higher HER2 expression and ERBB2 amplification are associated with immune-depleted features at baseline, whereas trastuzumab-containing preoperative

therapy is associated with remodeling of the tumor immune microenvironment in paired specimens (Fig. 4).

Discussion

The present study integrates pathologic, genomic, and paired treatment-associated analyses to characterize the immune context of HER2-expressing gastric cancer. Three findings are central. First, in treatment-naïve resected tumors, the IHC 2+/3+ group had lower CD8+ T-cell infiltration and lower PD-L1 CPS than the IHC 0/1+ group. Second, an independent TCGA analysis showed that ERBB2-amplified gastric cancers had lower somatic mutation counts, lower CYT scores, and lower estimated fractions of several immune-cell populations. Third, in a small paired cohort, trastuzumab-containing preoperative therapy was associated with increased CD8+ T-cell infiltration and increased PD-L1, PD-L2, and SIRPα, whereas CD80 did not significantly change. The concordance between the institutional and TCGA analyses supports an immune-depleted baseline phenotype in at least a subset of HER2-high/ERBB2-amplified gastric cancers, while the paired analysis suggests that treatment can shift this phenotype toward a more inflamed state.

HER2 expression and an immune-depleted baseline phenotype

The lower CD8+ T-cell density and lower PD-L1 CPS in tumors with higher HER2 expression are consistent with a relative paucity of ongoing antitumor T-cell activity. The TCGA findings reinforce this interpretation. ERBB2-amplified tumors had lower CYT scores, indicating reduced expression of the cytolytic effector genes GZMA and PRF1, and lower estimated CD8+ T-cell fractions. They also had lower somatic mutation counts. A lower mutation count may, on average, reduce the number of potential neoantigens, although neoantigen quantity was not directly measured here and this relationship should not be interpreted deterministically. Importantly, prior gastric cancer studies have also reported reduced immune-cell infiltration in HER2-overexpressing tumors and lower CD8+ T-cell infiltration in association with HER2-high tumor regions, providing independent context for the phenotype observed in our cohorts [16, 17]. Accordingly, the present data support an immune-depleted phenotype rather than proving a specific causal mechanism linking ERBB2 amplification to immune exclusion.

The CIBERSORT analysis also identified lower M2 macrophages, resting mast cells, memory B cells, and activated CD4+ memory T cells in ERBB2-amplified tumors. The reduction in M2 macrophages, despite their commonly immunosuppressive role, illustrates why an immune-cold tumor should not be equated simply with accumulation of suppressive cells. An immune-desert phenotype can instead reflect broadly reduced leukocyte recruitment across both effector and regulatory compartments. This broader interpretation is more consistent with the pattern observed in the current dataset.

Treatment-associated immune remodeling

In the paired cohort, post-treatment specimens contained more CD8+ T cells and showed higher PD-L1 and PD-L2 expression. One plausible explanation is that trastuzumab-mediated ADCC and treatment-

induced tumor-cell injury increase antigen release and local inflammatory signaling, which in turn enhance recruitment or activation of cytotoxic T cells [8–10]. A recent study of HER2-positive gastric cancer similarly reported treatment-associated changes in immune-cell infiltration after the addition of trastuzumab to preoperative chemotherapy, including increased CD8 + T-cell infiltration in paired specimens [18]. Increased checkpoint-ligand expression, particularly PD-L1, can occur as part of adaptive immune resistance in response to interferon-driven inflammation [19]. Thus, greater PD-L1 expression after treatment does not necessarily indicate a more immunosuppressed tumor; in the context of increased CD8 infiltration, it may instead mark an activated but adaptively restrained immune microenvironment.

SIRPα-positive cells also increased after treatment. SIRPα is expressed predominantly on myeloid cells and transmits an inhibitory phagocytic signal when engaged by CD47 [20]. The observed increase therefore suggests treatment-associated remodeling of the myeloid compartment, although the current IHC approach cannot determine the precise myeloid cell subsets involved or establish functional activation of the CD47-SIRPα axis. CD80, in contrast, did not significantly change, indicating that immune remodeling was selective rather than a uniform increase across all immune-related markers.

Implications for HER2-directed therapy plus immune checkpoint blockade

The clinical relevance of these findings is underscored by KEYNOTE-811, in which pembrolizumab added to trastuzumab and chemotherapy improved outcomes in HER2-positive advanced gastric/GEJ cancer [7]. The current study did not evaluate response to ICIs directly and therefore cannot establish that the observed tissue changes mediate clinical benefit from PD-1 blockade. Nevertheless, the data provide a plausible biological sequence: some HER2-high tumors may begin with limited T-cell infiltration, while HER2-directed treatment and concomitant tumor injury may increase local immune-cell infiltration and induce PD-1/PD-L1-axis signaling. Such treatment-induced inflammation could create a setting in which checkpoint blockade becomes more effective.

This distinction is important for interpretation. The data do not support the broad claim that all HER2-positive gastric cancers are intrinsically immune-cold, nor that trastuzumab alone converts every tumor from cold to hot. HER2 expression is heterogeneous in gastric cancer, and HER2 status may change after trastuzumab exposure [4, 21]. The institutional IHC grouping was exploratory, and the paired cohort received trastuzumab-containing treatment rather than a controlled trastuzumab monotherapy intervention. The most defensible conclusion is therefore that higher HER2 expression/ERBB2 amplification was associated with immune-depleted features in the analyzed cohorts, and that trastuzumab-containing treatment was associated with immune remodeling in paired tissue samples.

Limitations

Several limitations should be acknowledged. First, both institutional cohorts were small, particularly the paired pre-/post-treatment cohort of six patients, limiting statistical precision and precluding meaningful subgroup analyses. Second, CD8 and checkpoint-marker evaluation by conventional IHC is sensitive to

sampling, fixation, and scoring methodology, and hotspot-based assessment does not capture full spatial heterogeneity. Multiplex IHC, digital pathology, or spatial transcriptomic approaches would enable more detailed mapping of HER2-expressing tumor clones and neighboring immune populations. Third, TCGA analysis was cross-sectional and depended on platform-specific copy-number and transcriptomic data; the results should therefore be regarded as external supportive evidence rather than a direct validation cohort. Fourth, the institutional IHC analysis grouped IHC 2 + and 3 + tumors together without documented ISH confirmation for all IHC 2 + cases. This operational grouping reflects protein-expression intensity and should not be interpreted as equivalent to the clinical definition of HER2-positive gastric cancer. Finally, the study did not directly assess ICI response or survival according to treatment-associated immune changes. Prospective studies linking paired TME profiling to clinical outcome after HER2-directed therapy plus ICI are warranted.

Conclusion

Higher HER2 protein expression and ERBB2 amplification were associated with an immune-depleted tumor microenvironment characterized by lower CD8+ T-cell infiltration and reduced cytolytic activity. In contrast, trastuzumab-containing preoperative therapy was associated with increased CD8 infiltration and increased PD-L1, PD-L2, and SIRPα expression in paired specimens. These observations support a model in which HER2-directed treatment can be accompanied by immune remodeling and provide a biologic rationale for therapeutic combinations of anti-HER2 agents and immune checkpoint blockade. The findings should be validated in larger cohorts with standardized HER2 definitions, spatial immune profiling, and direct correlation with treatment response.

Declarations

Author Contribution

T.H. conceived and designed the study, performed the experiments and analyses, interpreted the data, and drafted the manuscript. Q.H. performed the TCGA analysis and contributed to data interpretation. T.K., S.N., Y.T., and T.N. contributed to the acquisition and interpretation of clinical and pathological data and critically reviewed the manuscript. K.A. and E.O. supervised the study, contributed to the study design and interpretation of the data, and critically revised the manuscript. T.Y. provided overall academic supervision and critically reviewed the manuscript. All authors reviewed and approved the final manuscript.

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Figures

Figure1(A)

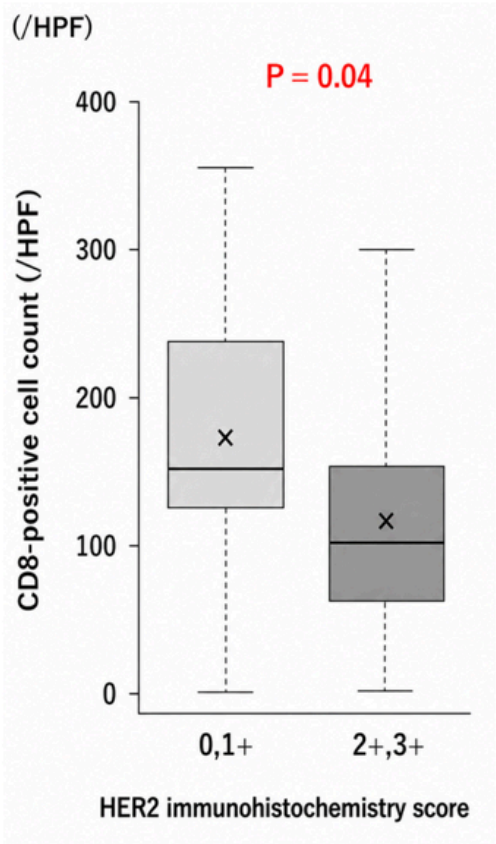


Figure1(B)

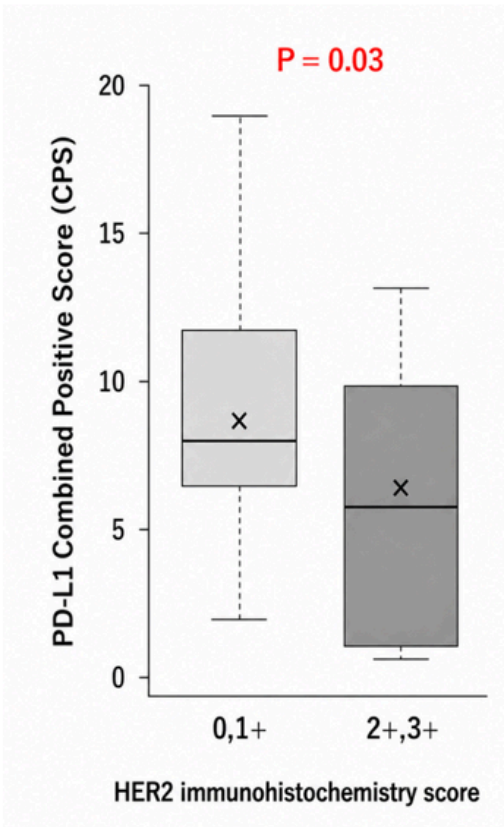


Figure 1

A Association between HER2 IHC category and CD8+ T-cell infiltration. CD8+ cells were counted in five ×400 hotspots at the invasive front and averaged as cells/HPF. Tumors were grouped as HER2 IHC 0/1+

and IHC 2+/3+. The IHC 2+/3+ group showed significantly lower CD8+ T-cell infiltration (P=0.04).

1B Association between HER2 IHC category and PD-L1 combined positive score (CPS). PD-L1 was evaluated using clone 28-8. The IHC 2+/3+ group showed significantly lower PD-L1 CPS than the IHC 0/1+ group (P=0.03).

Figure2(A)

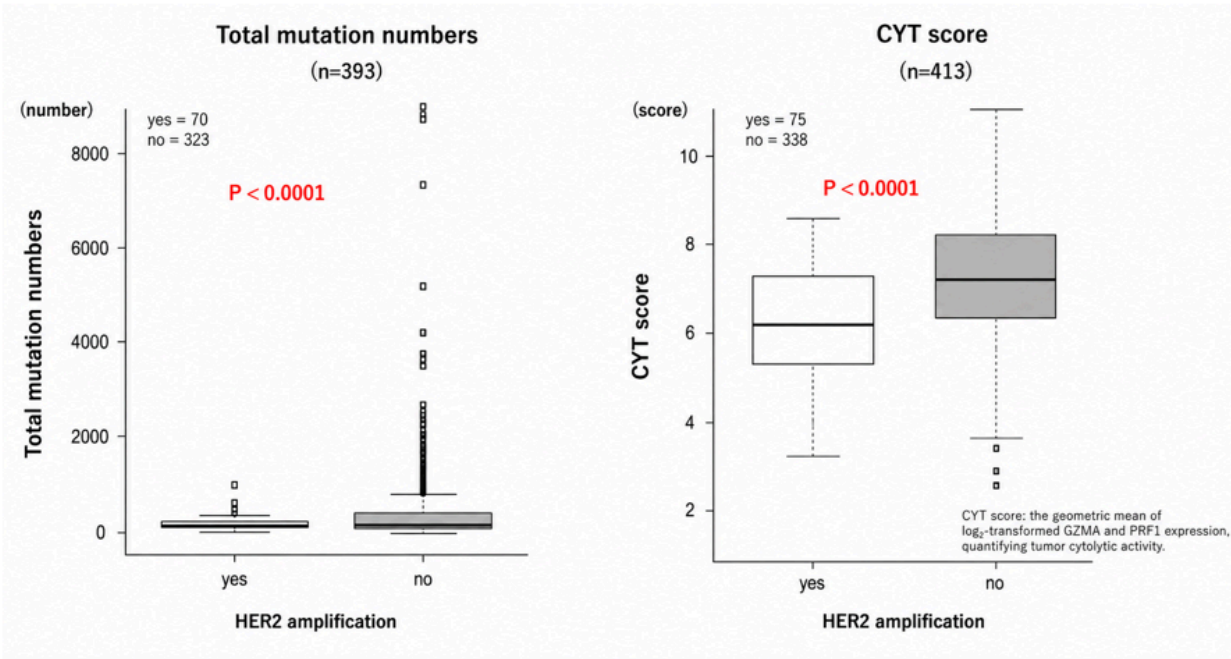


Figure2(B)

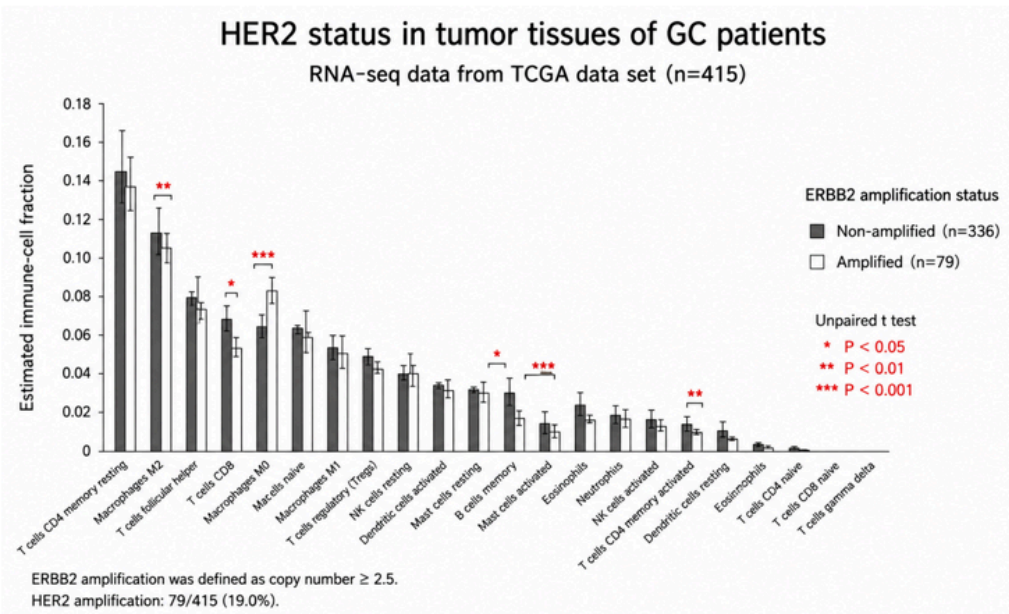


Figure 2

A ERBB2 amplification in relation to somatic mutation count (left; n=393) and CYT score (right; n=413) in TCGA gastric cancer. ERBB2-amplified tumors showed lower values for both measures ($P < 0.0001$).

B CIBERSORT-estimated immune-cell composition according to ERBB2 amplification status in TCGA gastric cancer (n=415). Significant differences are indicated in the original figure. ERBB2-amplified tumors had lower estimated fractions of several immune-cell subsets, including CD8+ T cells.

Figure3

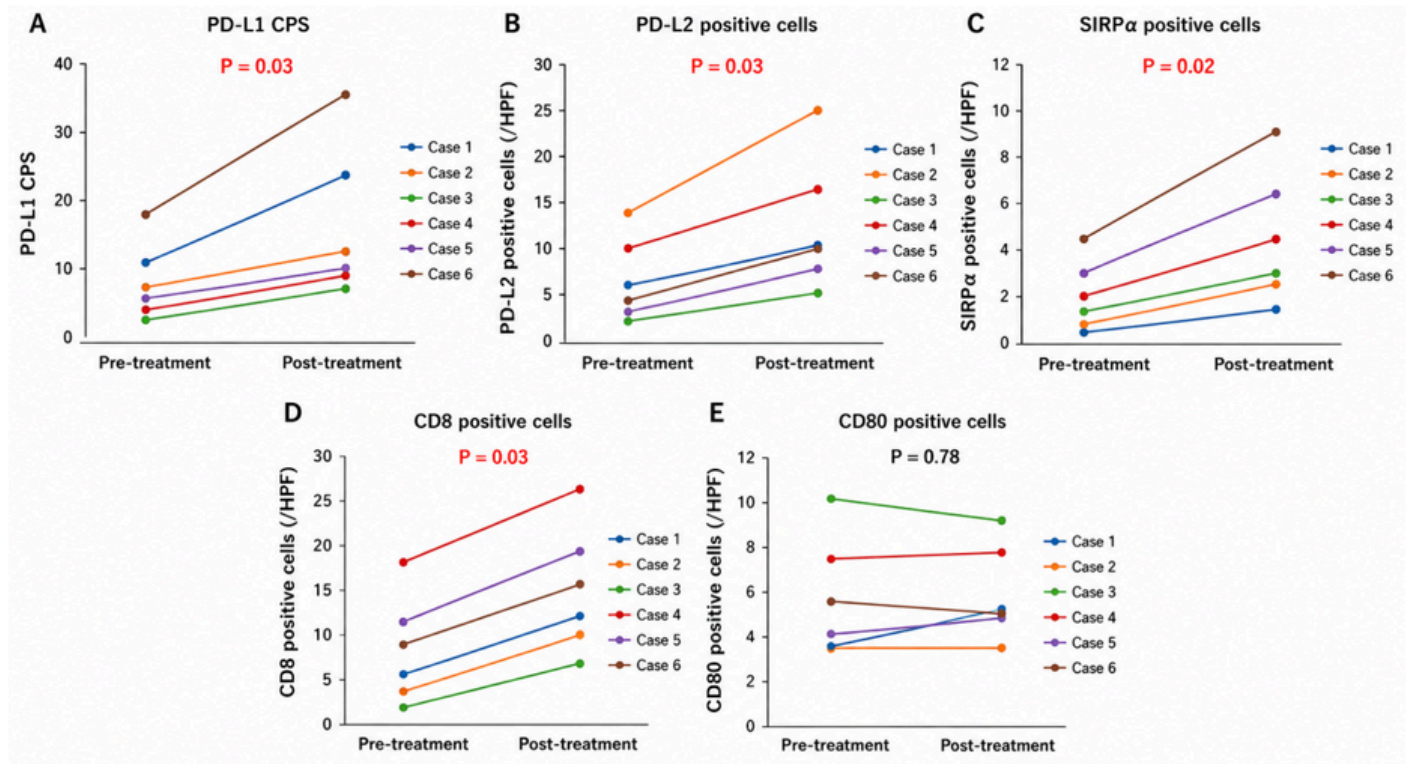


Figure 3

Changes in immune markers before and after trastuzumab-containing preoperative therapy in six patients with HER2-positive gastric cancer. Paired pretreatment biopsy and post-treatment resection specimens were assessed for (A) PD-L1 combined positive score (CPS), (B) PD-L2-positive cells, (C) SIRPα-positive cells, (D) CD8-positive cells, and (E) CD80-positive cells. Each line connects the pretreatment and post-treatment values from the same patient. PD-L1 CPS, PD-L2-positive cells, SIRPα-positive cells, and CD8-positive cells increased significantly after treatment, whereas CD80-positive cells did not change significantly. P values were calculated using the Wilcoxon signed-rank test.

Figure4

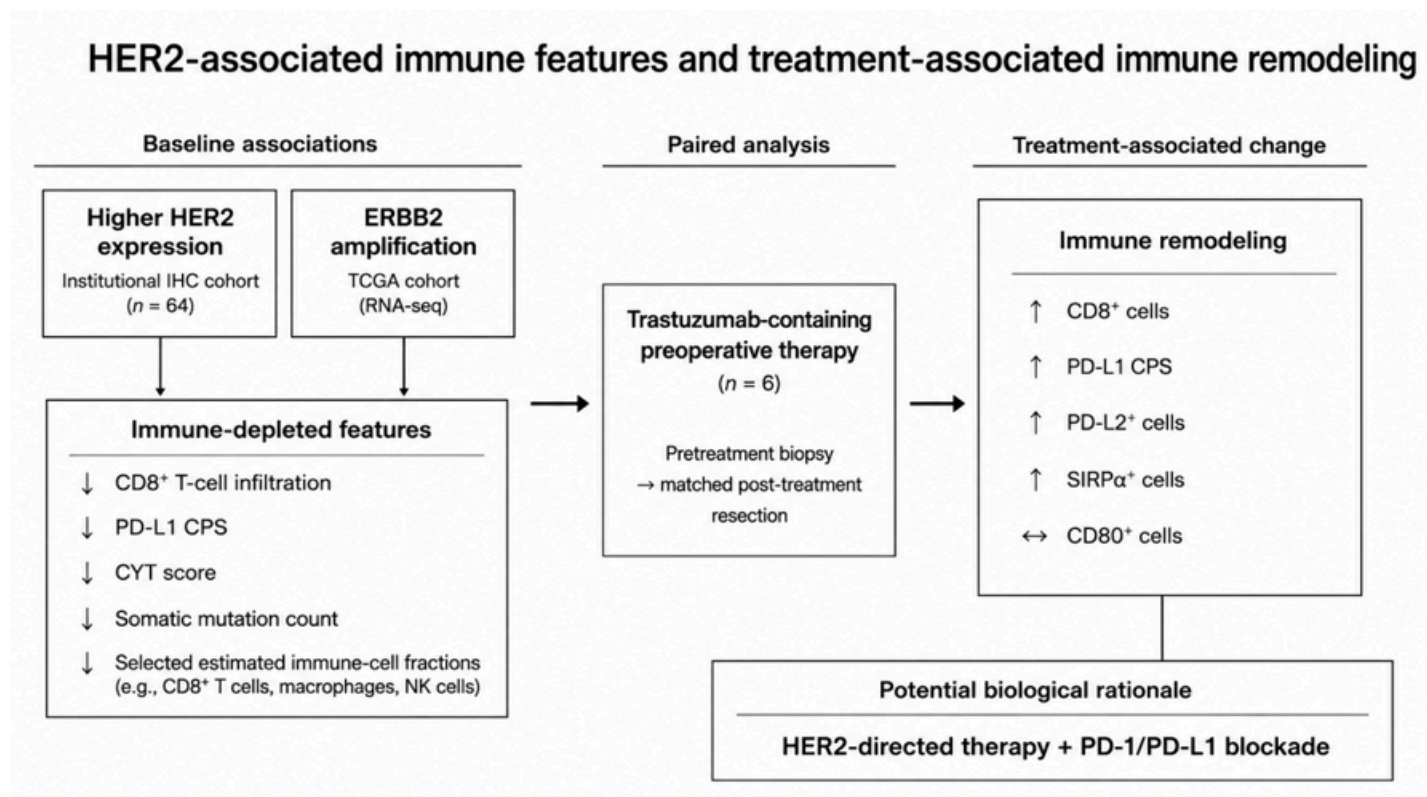


Figure 4

Graphical summary of HER2-associated immune features and treatment-associated immune remodeling. Higher HER2 expression in the institutional cohort and ERBB2 amplification in the TCGA cohort were independently associated with immune-depleted features. In paired specimens from six patients, trastuzumab-containing preoperative therapy was associated with increased CD8-positive cells, PD-L1 CPS, PD-L2-positive cells, and SIRPα-positive cells, whereas CD80-positive cells did not significantly change. These findings provide a potential biological rationale for combining HER2-directed therapy with PD-1/PD-L1 blockade. The associations illustrated in this schematic do not establish a direct causal mechanism.

Supplementary Files

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