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ORIGINAL ARTICLE

# Clinicopathologic characteristics and genomic profiling of HER2-low advanced gastric or gastroesophageal junction cancer

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**Background:** Human epidermal growth factor 2 (HER2)-low expression has recently emerged as a potential therapeutic target with the advent of HER2-directed antibody—drug conjugates. However, the clinicopathologic and molecular features of HER2-low advanced gastric or gastroesophageal junction (G/GEJ) cancer remain inadequately characterized.

**Patients and methods:** We retrospectively analyzed 2007 patients with stage IV G/GEJ cancer treated between 2015 and 2022 at Yonsei Cancer Center, Korea. HER2 status was classified as HER2-high (immunohistochemistry [IHC] 3+ or IHC 2+/*in situ* hybridization [ISH]+), HER2-low (IHC 2+/ISH− or IHC 1+) and HER2-null (IHC 0). Clinicopathologic features and survival outcomes were assessed in patients receiving first-line doublet chemotherapy with or without immune checkpoint inhibitors (ICIs). For molecular analyses, pretreatment tumors from 777 patients underwent in-house next-generation sequencing (NGS), excluding Epstein—Barr virus (EBV)—positive and microsatellite instability (MSI)—high cases.

**Results:** Among 2007 patients, 372 (18.5%) were HER2-high, 523 (26.1%) were HER2-low, and 1112 (55.4%) were HER2-null. HER2-low tumors closely resembled HER2-null tumors in histopathology, EBV status, MSI, and programmed death-ligand 1 status. In the survival analysis cohort ($n = 1417$), first-line progression-free survival was 8.0 months (HER2-high), 6.0 months (HER2-low), and 6.1 months (HER2-null), while overall survival was 17.2, 13.4, and 14.5 months, respectively. Combination with ICIs conferred greater survival benefit in HER2-low tumors compared with HER2-null tumors. In the NGS cohort ($n = 777$), HER2-low tumors were significantly enriched for angiogenesis pathway alterations, which were associated with worse survival, a pattern not observed in other subgroups.

**Conclusions:** HER2-low G/GEJ cancer represents a distinct biological subtype with intermediate survival outcomes and specific angiogenesis-related molecular features. These findings support the need for dedicated therapeutic strategies and further clinical research in HER2-low G/GEJ cancer.

**Key words:** HER2-low, gastric cancer, gastroesophageal junction cancer, immunotherapy, molecular profiling

## INTRODUCTION

Gastric and gastroesophageal junction (G/GEJ) cancer is the second leading cause of cancer-related deaths and the fifth most common cancer worldwide, and Eastern Asia has one of the highest incidence rates.[1] Doublet chemotherapy with platinum and fluoropyrimidines, with or without immune checkpoint inhibitors (ICIs), is the recommended palliative first-line therapy for patients with advanced G/GEJ cancer,[2-4] and patients with human epidermal growth factor 2 (HER2)—positive or claudin-18.2—positive tumors benefit from targeted agents trastuzumab[5-7] or zolbetuximab,[8,9] respectively, as the first-line therapy.

For patients with HER2-overexpressing locally advanced or metastatic G/GEJ cancer and a tumor score of 3+ by immunohistochemistry (IHC), or IHC 2+ and evidence of HER2 amplification by *in situ* hybridization (ISH), which account for only ∼15% of G/GEJ cancers,[10,11] the addition of trastuzumab to first-line chemotherapy demonstrated overall survival (OS) benefit over chemotherapy alone in the ToGA trial[5] and became standard of care for over a

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decade. Recently, the KEYNOTE-811 trial showed prolonged progression-free survival (PFS) and OS with pembrolizumab added to trastuzumab plus chemotherapy among programmed death-ligand 1 (PD-L1)-positive subgroup of patients with HER2-positive G/GEJ cancer.[6,7,12]

Recent advances in HER2-targeting agents, including HER2 antibody—drug conjugates (ADCs) have shown promising results in targeting HER2-low (HER2 IHC 2+/ISH− or HER2 1+) expressing cancers. In breast cancer, trastuzumab deruxtecan (T-DXd), an ADC comprising a humanized anti-HER2 monoclonal antibody and a cytotoxic topoisomerase I inhibitor, enhanced OS and PFS for pretreated HER2-low hormone receptor—positive breast cancer over physician's choice treatment in the phase III DESTINY-Breast04 trial,[13] which became the first approved treatment for HER2-low expressing cancers. In G/GEJ adenocarcinoma, the randomized phase II DESTINY-Gastric01 study showed benefit of T-DXd for patients with HER2-positive G/GEJ cancer in comparison with those treated with the chemotherapy of physician's choice.[14] In the exploratory cohort of patients with HER2 IHC 2+/ISH− and HER2 1+ (HER2-low) G/GEJ adenocarcinoma, DESTINY-Gastric01 found an objective response rate of 26.3% and 9.5%, respectively,[15] showing potential benefit of anti-HER2 ADCs for these HER2-low G/GEJ cancers. These findings have generated growing interest in HER2-low G/GEJ cancer as a potential biological and therapeutic entity. However, few studies have comprehensively compared HER2-low G/GEJ cancers with HER2-high (HER2-positive, HER2 3+ or 2+/ISH+) or HER2-null (HER2 IHC 0) tumors, in terms of survival outcomes and molecular characteristics.

To define comprehensive clinicopathologic characteristics and prognosis of patients with HER2-low advanced G/GEJ cancer, we retrospectively analyzed a large cohort of patients with G/GEJ cancer and further analyzed the survival of the patients treated with palliative doublet chemotherapy—based treatment according to HER2 status. Moreover, next-generation sequencing (NGS) was performed to explore molecular features specific to HER2-low tumors in comparison with HER2-high and HER2-null tumors.

## METHODS

### Design and patients

A retrospective cohort analysis was performed at Yonsei Cancer Center, South Korea. Based on a review of electronic medical records, we identified histologically confirmed patients with stage IV advanced G/GEJ cancer with known HER2 status. Data with the following variables were retrospectively collected and analyzed: age at the beginning of palliative treatment, sex, pathology, histology according to Lauren classification, Eastern Cooperative Oncology Group performance status, previous history of surgery, metastatic organ, treatment regimens and outcomes (response and survival).

The study was reviewed and approved by the institutional review and ethics board of Severance Hospital, and the requirement for informed consent was waived owing to the retrospective nature of the study.

### IHC and ISH

Tumor tissues were fixed in 10% formalin, embedded in paraffin, and cut into 4 μm-thick tissue sections for further analyses. IHC staining was performed using the Ventana Benchmark XT automated staining system (Ventana Medical Systems; Tucson, AZ) according to the manufacturer's protocol. To assess HER2 status, anti-HER2/neu antibody (clone 4B5; Ventana Medical Systems) was used for IHC, and the HER2 expression scoring system was applied according to the American Society of Clinical Oncology/College of American Pathologists guidelines for HER2 testing in G/GEJ cancer.[16] In addition, *HER2* DNA amplification was evaluated using the silver ISH method, which was performed using the INFORM® *HER2* DNA and chromosome 17 (*CEP17*) probes (Ventana Medical Systems) with a Ventana Benchmark XT automated staining system, according to the manufacturer's instructions. *HER2* DNA amplification was defined as HER2/CEP17 ratio of ≥2.0. Epstein—Barr virus (EBV) status was assessed using EBV-encoded small RNA ISH following standard protocols. The INFORM® EBER Probe (Ventana Medical Systems) was used to perform automated staining, according to the manufacturer's instructions. Positive staining was defined by diffuse staining of tumor cells. Antibodies against MLH1 (clone M1; Ventana Medical Systems), MutS protein homolog 2 (MSH2; clone G219-1129; Cell Marque, Rocklin, CA), PMS2 (clone MRQ-28; 1:40; Cell Marque), and MutS homolog 6 (MSH6; clone 44; 1:100; Cell Marque) were used to define mismatch repair status, as previously described.[17] Proficient mismatch repair was defined as positive staining of all stained mismatch repair genes, where deficient mismatch repair (dMMR) was defined as loss of IHC expression of at least one of the MMR proteins. Microsatellite instability (MSI) status of some patients were analyzed by PCR, resulting MSI-high (MSI-H; tumor with two or more unstable markers) or microsatellite stable (cases with no unstable markers). PD-L1 protein expression was determined with a combined positive score (CPS) using the Dako PD-L1 IHC 22C3 pharmDx kit (Agilent, Santa Clara, CA).

### Efficacy outcomes

For the survival analyses, only patients who were treated with regimens containing 5-fluorouracil and platinum-based doublet chemotherapy as palliative first-line treatment were included. For patients with G/GEJ cancer, both measurable or evaluable but nonmeasurable disease per RECIST version 1.1, efficacy outcomes including PFS and OS were measured. PFS was defined as the time from palliative first-line treatment initiation to the date of disease progression or death from any cause. For patients without disease progression or those who did not die, the censoring date was the last date nearest to their last response evaluation. OS was defined as the time from palliative first-line treatment initiation to death from any cause. The OS

censoring date was the last date of the analysis data cut-off when the participant was known to be alive.

### Tissue genomic analyses

Tumor tissues from either primary or metastatic tumors were obtained. Quality-controlled samples were subjected to NGS by TruSight Oncology 500 (Illumina, San Diego, CA) or in-house panel sequencing using the CancerMaster Panel V2,[18] which covers 524 genes for single nucleotide variants, 143 for copy-number variations, and 18 for fusions. The process for CancerMaster in-house NGS has been previously described.[19] Tumor mutation burden (TMB; mutations per megabase) was estimated as the total number of detected nonsynonymous mutations (single nucleotide polymorphism variant allele frequency [VAF] >5% and indel VAF >10%; putative germline mutations reported in population databases [Korean Variant Archive,[20] Korean Reference Genome Database,[21] The Exome Aggregation Consortium (East Asian)[22] and 1,000 Genomes Phase 3 (East Asian)][23] were removed divided by the length of the covered coding regions. The genomic landscape plot (oncoplots) was generated using the R package 'maftools.'[24] Signaling pathways were annotated manually according to previously reported curated oncogenic signaling pathways.[25] For NGS-based molecular profiling investigation, EBV-positive or MSI-H tumors were excluded.

### Statistical analysis

Continuous variables were compared between groups using an independent samples t-test (Student's *t*-test) for normally distributed data, whereas non-normally distributed variables were analyzed using the Wilcoxon rank-sum test. Descriptive statistics for categorical variables, including baseline characteristics and toxicities, were summarized as counts and percentages, and comparisons were made using the Chi-square test or Fisher's exact test. The Kaplan—Meier method was used to estimate the OS and PFS. The log-rank test was applied to determine the difference in survival between the groups. The Cox proportional hazards model was used to assess the significant prognostic factors associated with OS and PFS, using hazard ratios (HRs) and 95% confidence intervals (CIs). When comparing oncogenic pathway alteration, patients were considered to have a pathway alteration if at least one gene from our curated pathway list was mutated. To evaluate whether pathway alterations were significantly prevalent, odds ratios (ORs) and 95% CIs were calculated from logistic regression. Statistical significance was assessed using Wald tests. Results were visualized using forest plots to display the ORs and CIs for each comparison. Statistical analyses were performed using SAS software 9.4 (SAS Institute, Inc., Cary, NC), R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria), and GraphPad Prism (version 8; GraphPad Software, San Diego, CA). Two-sided $P$ values $< 0.05$ were considered statistically significant.

## RESULTS

### Prevalence and clinicopathologic findings of HER2-low G/GEJ cancer

A total of 2396 consecutive patients with G/GEJ cancer who were diagnosed as stage IV between January 2015 and December 2022, were screened. Among them, 356 patients without known HER2 IHC status were excluded from the epidemiology analysis (Figure 1). Among the 2007 patients included in the study as the epidemiology analysis dataset, 372 (18.5%) were classified as HER2-high, comprising 312 patients with HER2 IHC 3+ and 60 patients with HER2 IHC 2+/ISH+. Additionally, 523 patients (26.1%) were categorized as HER2-low, with 76 patients exhibiting HER2 IHC 2+/ISH− and 447 patients expressing HER2 IHC 1+. Notably, most patients ($n = 1112$, 55.4%) were categorized as HER2-null (HER2 IHC 0). In the comparison of clinicopathologic features across HER2-expressing groups (Table 1; Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2026.106963), HER2-high patients were more often male (73.7%) and older at diagnosis, and had the highest proportion of intestinal-type histology (66.4%). In contrast, the HER2-null group had the highest frequency of poorly cohesive carcinoma or signet ring cell features 27.8%) and peritoneal metastasis (69.0%). The HER2-low group exhibited intermediate features, with the highest prevalence of prior gastrectomy (42.6%) and a lower frequency of lymph node metastases compared with the other subgroups. Overall, HER2-low G/GEJ cancer demonstrated a heterogeneous profile, sharing similarities with both HER2-high and HER2-null tumors, but also displaying distinct characteristics.

We then analyzed the distribution of well-known biomarkers (i.e. EBV-associated, PD-L1 CPS ≥1, and dMMR/MSI-H) according to HER2 expression status (Table 1, Figure 2). Among patients with available PD-L1 IHC results, prevalence of PD-L1—positive patients (PD-L1 CPS ≥1) were most common among HER2-high patients (57.3%), followed by HER2-low (51.9%) and HER2-null (48.2%). Prevalence of EBV-associated patients were similar between the three groups (3.1%-3.7%). Interestingly, the HER2-low group had more dMMR/MSI-H patients (6.3%) compared with the HER2-null (5.5%) or HER2-high (1.2%) groups.

### Survival analysis

After excluding 590 patients, 1417 patients were included as the survival analysis dataset (Figure 1). At the time of data cut-off (4 October 2024), the median follow-up duration was 54.8 months (95% CI 47.7-58.5). HER2-low ($n = 367$) patients showed similar survival as HER2-null ($n = 759$) but shorter survival compared with HER2-high ($n = 291$), in terms of PFS of palliative first-line 5-fluorouracil/platinum doublet-based chemotherapy (6.0 versus 6.1 versus 8.0 months, log-rank $P = 0.0006$; Figure 3A) and OS (13.4 versus 14.5 versus 17.2 months, log-rank $P = 0.051$; Figure 3B). HER2-low patients exhibited the shortest first-line median PFS (6.0 months) and OS

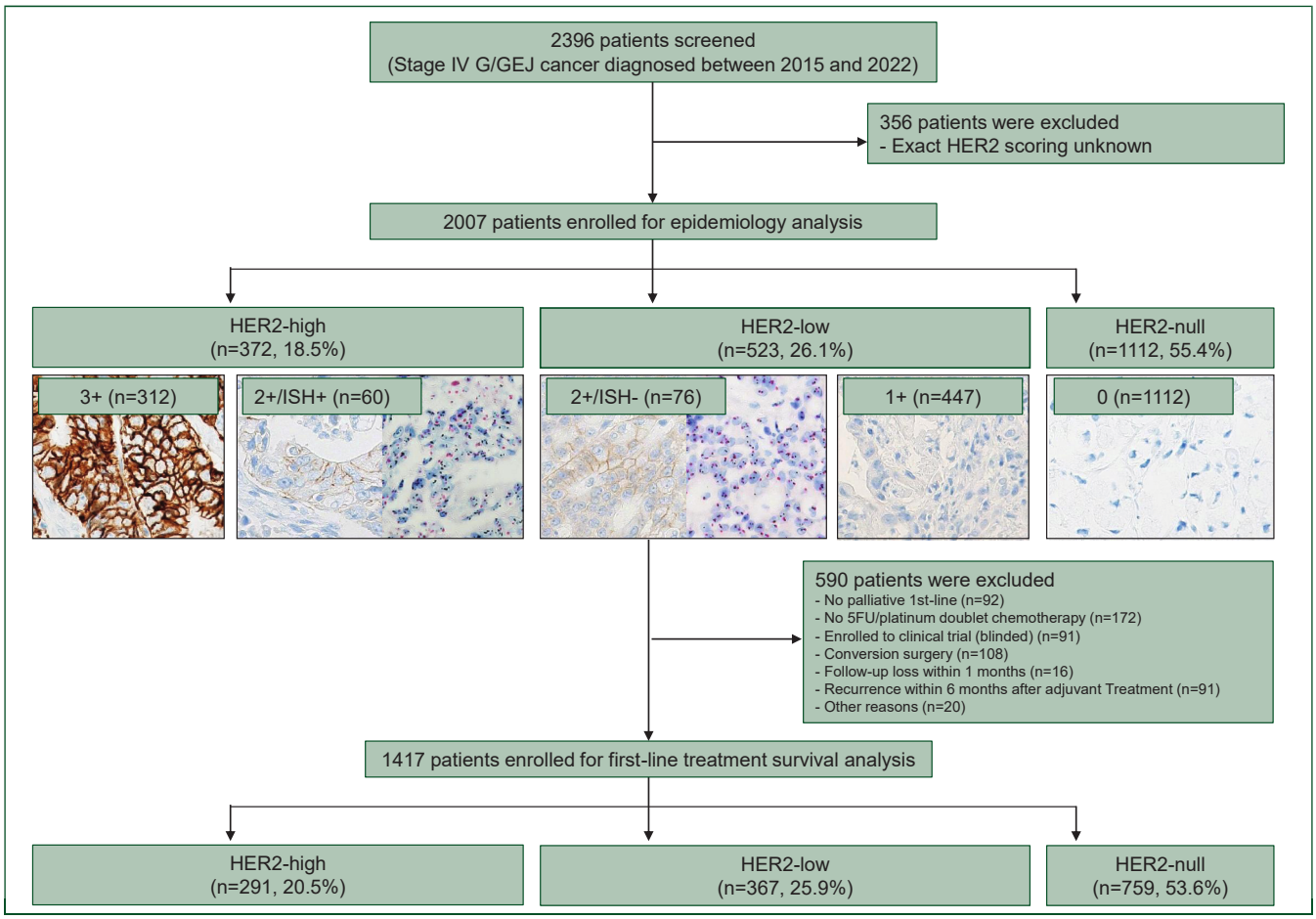


**Figure 1. Graphic representation of the study design and Consolidated Standards of Reporting Trials diagram.**
5FU, 5-fluorouracil; G/GEJ, gastric or gastroesophageal junction; ISH, *in situ* hybridization; HER2, human epidermal receptor 2.

(13.4 months) compared with the other two groups. As current first-line standard of care includes ICI combination for PD-L1—positive HER2-negative G/GEJ cancers, we further analyzed survival benefit of ICI addition to first-line doublet chemotherapy according to HER2 status. Compared with chemotherapy alone, addition of ICI provided greater improvement in terms of PFS (HR 0.53, 95% CI 0.37-0.76, $P = 0.0005$) and OS (HR 0.54, 95% CI 0.67-1.02, $P = 0.08$) in HER2-low tumors compared with HER2-null tumors (PFS: HR 0.83, 95% CI 0.67-1.02, $P = 0.0803$ and OS: HR 0.86, 95% CI 0.68-1.10, $P = 0.23$) (Figure 3C and D). Survival among HER2-low patients according to HER2 status (HER2 IHC 2+/ISH− versus HER2 IHC 1+) seemed similar (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2026.106963).

### *Molecular profiling according to HER2 status*

Among the epidemiology analysis dataset, excluding EBV-positive or MSI-H tumors, 777 patients had available targeted panel sequencing results. Curated pathways and selected genes altered in ≥5% of the patients according to HER2 status and correlated clinicopathologic features are shown as an oncoplot in Figure 4A. A forest plot showing pathway alterations per HER2-expression groups and their ORs with 95% CIs comparing HER2-null with HER2-low, HER2-null HER2-high, and HER2-low versus HER2-high are shown in Figure 4B. HER2-high tumors had more altered genes related to cell cycle, NOTCH, RTK/RAS, or TP53 pathways, compared with HER2-low or HER2-null groups. HER2-high group also showed more altered genes related to immune-related pathways compared to HER2-low group. Meanwhile, the HER2-low group showed more gene alterations related to DNA damage response and phosphoinositide 3-kinase pathways compared with HER2-null group. Interestingly, genes related to angiogenesis were significantly altered in the HER2-low group compared with HER2-high (OR 2.329, 95% CI 1.441-3.760, $P < 0.001$) or HER2-null (OR 1.450, 95% CI 1.045-2.021, $P = 0.028$) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2026.106963). Among 777 patients, 578 patients were available for survival analysis. Patients with angiogenesis pathway alteration ($n = 73$) had shorter OS compared with patients without ($n = 87$) (13.7 versus 20.1 months, HR 1.63, 95% CI 1.15-2.32, $P = 0.0067$) among the HER2-low group (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2026.106963). In contrast, patients with an altered angiogenesis pathway had similar OS compared with patients without, among HER2-null and HER2-high groups.

**Table 1. Baseline patient characteristics (epidemiology analysis data set, $N = 2007$)**

| Characteristic | Total ($N = 2007$) | HER2-null ($n = 1112$) | HER2-low ($n = 523$) | HER2-high ($n = 372$) | *P* value |
|---|---|---|---|---|---|
| Age (years), median (IQR) | 60 (51-68) | 59 (49-68) | 60 (51-68) | 62 (54-69) | 0.0010 |
| Age, *n* (%) | | | | | 0.0426 |
| ≥65 years | 699 (34.8) | 369 (33.2) | 180 (34.4) | 150 (40.3) | |
| <65 years | 1308 (65.2) | 743 (66.8) | 343 (65.6) | 222 (59.7) | |
| Sex, *n* (%) | | | | | < 0.0001 |
| Male | 1239 (61.7) | 639 (57.5) | 326 (62.3) | 274 (73.7) | |
| Female | 768 (38.3) | 473 (42.5) | 197 (37.7) | 98 (26.3) | |
| ECOG PS, *n* (%) | | | | | 0.5209 |
| 0 | 1466 (75.2) | 823 (75.8) | 374 (73.9) | 269 (75.3) | |
| 1 | 398 (20.4) | 217 (20.0) | 113 (22.3) | 68 (19.1) | |
| ≥2 | 85 (4.4) | 46 (4.2) | 19 (3.7) | 20 (5.6) | |
| Pathology group, *n* (%) | | | | | 0.0005 |
| Adenocarcinoma, well differentiated | 65 (3.3) | 17 (1.6) | 26 (5.1) | 22 (6.0) | |
| Adenocarcinoma, moderately differentiated | 553 (28.0) | 223 (20.4) | 156 (30.5) | 174 (47.3) | |
| Adenocarcinoma, poorly differentiated | 921 (45.9) | 544 (48.9) | 231 (44.2) | 146 (39.2) | |
| Signet ring cell/poorly cohesive carcinoma | 433 (21.6) | 309 (27.8) | 98 (18.7) | 26 (7.0) | |
| Others | 35 (1.7) | 19 (1.7) | 12 (2.3) | 4 (1.1) | |
| Histologic subtypes, *n* (%) | | | | | < 0.0001 |
| Intestinal | 255 (32.5) | 96 (21.8) | 82 (36.0) | 77 (66.4) | |
| Diffuse | 471 (60.0) | 304 (68.9) | 132 (57.9) | 35 (30.2) | |
| Mixed | 59 (7.5) | 41 (9.3) | 14 (6.1) | 4 (3.4) | |
| Previous gastrectomy, *n* (%) | | | | | < 0.0001 |
| Yes | 727 (36.2) | 411 (37.0) | 223 (42.6) | 93 (25.0) | |
| No | 1280 (63.8) | 701 (63.0) | 300 (57.4) | 279 (75.0) | |
| Number of metastatic organs, *n* (%) | | | | | < 0.0001 |
| 1 | 1326 (66.1) | 747 (67.2) | 370 (70.7) | 209 (56.2) | |
| 2 | 499 (24.9) | 272 (24.5) | 121 (23.1) | 106 (28.5) | |
| ≥3 | 182 (9.1) | 93 (8.4) | 32 (6.1) | 57 (15.3) | |
| Metastasis organ, *n* (%) | | | | | |
| Peritoneal | 1261 (62.8) | 767 (69.0) | 324 (62.0) | 170 (45.7) | < 0.0001 |
| Distant lymph node | 642 (32.0) | 334 (30.0) | 131 (25.0) | 177 (47.6) | < 0.0001 |
| Liver | 487 (24.3) | 220 (19.8) | 124 (23.7) | 143 (38.4) | < 0.0001 |
| Bone | 159 (7.9) | 88 (7.9) | 38 (7.3) | 33 (8.9) | 0.6811 |
| Lung | 136 (6.8) | 56 (5.0) | 33 (6.3) | 47 (12.6) | < 0.0001 |
| Ovary | 105 (5.2) | 68 (6.1) | 31 (5.9) | 6 (1.6) | 0.0024 |
| Brain | 15 (0.7) | 3 (0.3) | 7 (1.3) | 5 (1.3) | 0.0123[a] |
| EBV, *n* (%) | | | | | 0.7705 |
| Positive | 64 (3.4) | 39 (3.7) | 15 (3.1) | 10 (3.1) | |
| Negative | 1809 (96.6) | 1020 (96.3) | 474 (96.9) | 315 (96.9) | |
| MMR/MSI, *n* (%) | | | | | 0.0022 |
| dMMR/MSI-H | 94 (5.0) | 59 (5.5) | 31 (6.3) | 4 (1.2) | |
| pMMR/MSS | 1797 (95.0) | 1008 (94.5) | 465 (93.7) | 324 (98.8) | |
| PD-L1 22C3 CPS, *n* (%) | | | | | 0.0406 |
| ≥1 | 735 (50.5) | 418 (48.2) | 188 (51.9) | 129 (57.3) | |
| <1 | 720 (49.5) | 450 (51.8) | 174 (48.1) | 96 (42.7) | |
| Baseline CEA, *n* (%) | | | | | < 0.0001 |
| Elevated (≥5 mg/L) | 682 (36.8) | 325 (31.3) | 151 (31.8) | 206 (61.1) | |
| Within normal limit | 1170 (63.2) | 715 (68.7) | 324 (68.2) | 131 (38.9) | |
| Baseline CA19-9, *n* (%) | | | | | 0.0006 |
| Elevated (≥34 U/mL) | 594 (32.3) | 317 (30.7) | 140 (29.8) | 137 (41.3) | |
| Within normal limit | 1242 (67.7) | 717 (69.3) | 330 (70.2) | 195 (58.7) | |

ECOG performance status for 2.9% of patients ($n = 58$) are unknown. Histology subtype of 60.9% of patients ($n = 1222$) are unknown. EBV results are not reported for 134 (6.7%) patients. MMR/MSI results are not reported for 116 (5.8%) patients. PD-L1 22C3 CPS results are not reported for 552 (27.5%) patients. Baseline CEA results are not reported for 155 (7.7%) patients. Baseline CA19-9 results are not reported for 171 (8.5%) patients.

CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CPS, combined positive score; dMMR, deficient mismatch repair; ECOG, Eastern Cooperative Oncology Group; EBV, Epstein—Barr virus; HER2, human epidermal receptor 2; IQR, interquartile range; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability—high; MSS, microsatellite stable; pMMR, proficient mismatch repair; PD-L1, programmed death-ligand 1; PS, performance status.

[a]Fisher's exact test.

## DISCUSSION

In this large-scale retrospective cohort study, we identified HER2-low G/GEJ cancers, which accounted for approximately one-quarter of metastatic G/GEJ cancers, as a distinct subgroup with clinicopathologic features intermediate between HER2-high and HER2-null tumors. Interestingly, HER2-low G/GEJ cancers showed better survival when combined with first-line ICI compared with HER2-null tumors. Moreover, our molecular analyses revealed unique molecular features for HER2-low tumors compared with other subgroups, including an enriched angiogenesis pathway. These findings highlight the clinical relevance of HER2-low G/GEJ cancer as a

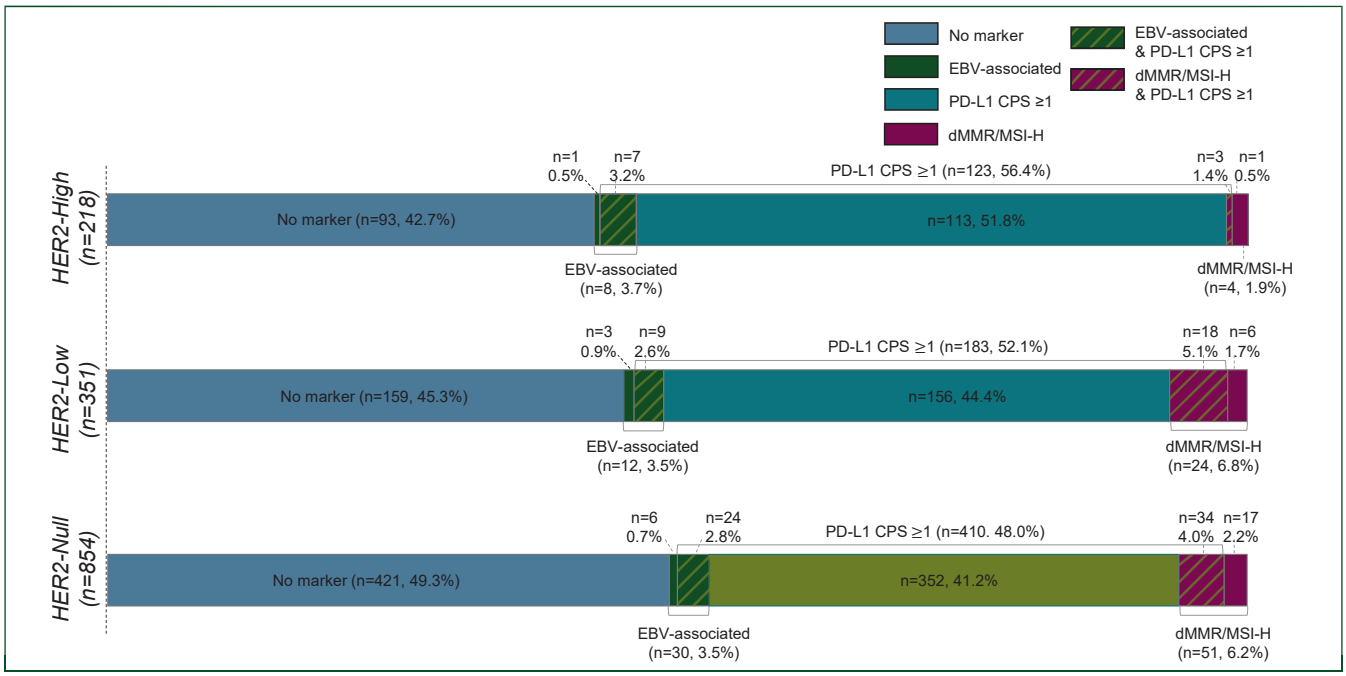


**Figure 2. Marker distributions by HER2 status.** Biomarker distributions by HER2 status among patients that have the results of all three biomarkers ($n = 1423$). CPS, combined positive score; dMMR, mismatch repair deficient; EBV, Epstein—Barr virus; HER2, human epidermal receptor 2; MSI-H, microsatellite instability—high; PD-L1, programmed death-ligand 1.

biologically and prognostically distinct entity, warranting further investigation into tailored therapeutic strategies beyond conventional HER2-targeted approaches.

Clinically, the three categorized HER2 expressing groups exhibited distinct clinicopathologic features, with the HER2-low group showing similarities to HER2-high in some aspects and to HER2-null in others. Interestingly, the HER2-low group had the highest prevalent history of gastrectomy (42.6%) compared with HER2-high (25.0%) or HER2-null (37.0%). Moreover, frequency of lymph node metastases was lowest in the HER2-low group compared with the other groups. Interestingly, the HER2-low group had more dMMR/MSI-H patients (6.3%) compared with the HER2-null (5.5%) or HER2-high (1.2%) groups.

When looking at survival from the initiation of first-line treatment, HER2-high patients exhibited the longest PFS and OS compared with HER2-low or HER2-null patients. This may by attributable to the usage of HER2-targeted therapies for HER2-high patients. As first-line therapy, anti—PD-1 ICIs are approved for HER2-negative G/GEJ cancer in combination with fluoropyrimidine-platinum chemotherapy; hence, we compared the effect of ICI between HER2-low or HER2-null patients. Interestingly, we observed that HER2-low patients derived greater relative benefit from the addition of ICIs to first-line chemotherapy, compared with HER2-null patients. This contrasts the data on breast cancer, as it is reported that HER2-low breast cancer is associated with a lower immune response compared with HER2-null breast cancer.[26] Our observation may be supported by the higher prevalence of PD-L1 positivity in HER2-low G/GEJ cancers and the increased frequency of dMMR/MSI-H status, suggesting a potentially more immunologically active tumor microenvironment. Also, in our additional analysis from the NGS cohort ($n = 777$), the HER2-low group had numerically higher TMB) compared with the HER2-null group (median 6.3 versus 5.81, $P = 0.074$). Proportion of patients with high-TMB (>20 mutations per megabase) was also numerically higher for the HER2-low group (7.0%) compared with HER2-null (4.3%) (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2026.106963). These findings suggest that HER2-low G/GEJ cancers may harbor a therapeutic vulnerability to immunotherapy, which warrants further prospective validation. However, given the non-randomized nature of this comparison and the potential confounding effects of differential PD-L1 and MMR status, these observations should be interpreted cautiously and considered hypothesis-generating.

HER2-low status was initially established in breast cancer, and accumulated epidemiologic evidence in HER2-low breast cancer resembles that seen in our HER2-low gastric cancer cohort—specifically, an intermediate but distinct profile between HER2-null and HER2-high tumors.[27] However, the prognostic impact is greatly inconsistent, as HER2-low breast cancers are typically marked by an immune-desert phenotype with minimal lymphocytic infiltration, which contrasts with the immune-active landscape seen in our patients with HER2-low gastric tumors.[26,28,29] These disparities reflect both intrinsic biological differences across tumor types and the heterogeneity within HER2-low classification itself, which in breast cancer spans hormone receptor—positive and triple-negative subtypes with distinct immune profiles. Thus, tissue-specific interpretation of HER2-low biology should be tailored to guide treatment strategies.[30] In HER2-low breast

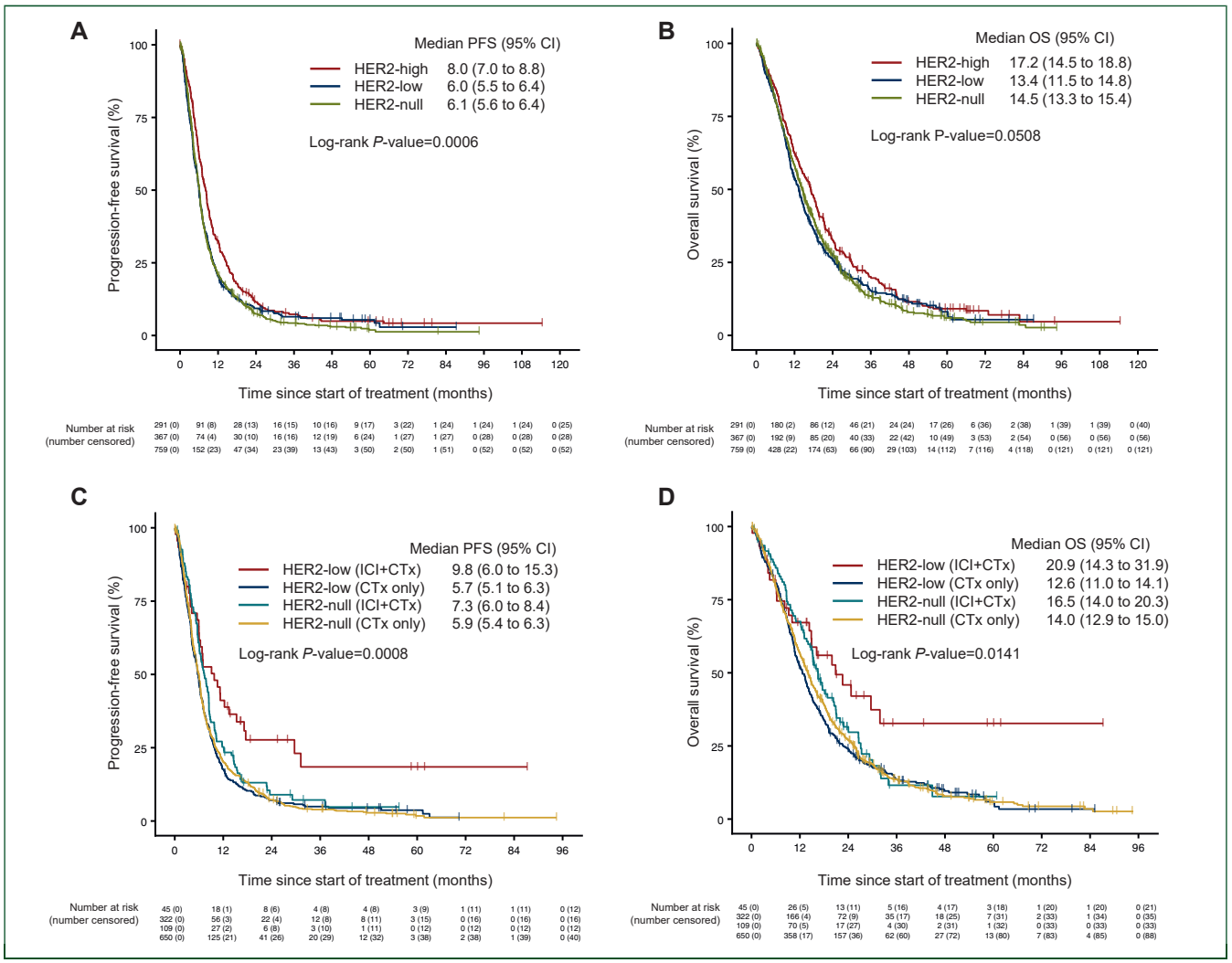


**Figure 3. Survival after the first-line treatment according to HER2 status and immune checkpoint inhibitor usage.** (A) Progression-free survival (PFS) and (B) overall survival (OS) of palliative first-line treatment according to HER2 status ($n = 1417$). First-line (C) PFS and (D) OS according to immune checkpoint inhibitor (ICI) usage among HER2-low/null patients ($n = 1176$). Median survival by months and 95% confidence intervals (CIs) are shown for each group or cohort. All *P* values were based on two-sided log-rank test.
CTx, chemotherapy; HER2, human epidermal receptor 2.

cancer, T-DXd enhanced PFS and OS for pretreated HER2-low hormone receptor—positive breast cancer over physician's choice treatment in the phase III DESTINY-Breast04 trial,[13] which became the first approved treatment for HER2-low expressing cancers. Supporting this hypothesis, exploratory data from DESTINY-Gastric01 demonstrated clinical efficacy of T-DXd in HER2-low G/GEJ cancers, implying the potential of anti-HER2 targeted therapies in patients with HER2-low G/GEJ cancer.[15] Currently, ongoing DESTINY-Gastric05 trial (NCT06731478) includes an exploratory cohort of patients with HER2-low gastric cancer to investigate the potential efficacy of first-line T-DXd combination in this subgroup. In addition, ongoing clinical trials targeting HER2-low G/GEJ cancer are investigating next-generation ADCs or bispecific T-cell engagers, the results of which are anticipated to be of high clinical interest.[31]

Our molecular profiling revealed additional insights. HER2-high tumors demonstrated enrichment of alterations in cell cycle, RTK/RAS, TP53, and NOTCH pathways, consistent with prior data on their aggressive biology.[10,32] In contrast, HER2-low tumors showed unique enrichment of angiogenesis-related gene alterations compared with other subgroups. The prognostic impact of angiogenesis alterations appeared to differ by HER2 status, with a significantly worse OS uniquely among HER2-low tumors, highlighting the need for therapeutic strategies targeting this pathway in this subgroup. Previously, a single-arm phase II study has shown benefit of adding trastuzumab to second-line anti-VEGFR2 ramucirumab plus paclitaxel for HER2-positive G/GEJ cancer.[33] One of the suggested resistance mechanisms to anti-HER2 treatment is upregulation of VEGF-A and transcriptional reprogramming toward angiogenesis.[33] These data collectively provide a rationale for combination approaches involving novel HER2-directed therapies and antiangiogenic agents in HER2-low G/GEJ cancers. Consequently, a phase Ib/II trial (NCT05894824) evaluating efficacy and safety of T-DXd in combination with ramucirumab in HER2-low tumors is ongoing. Outcomes from this trial are anticipated to further refine treatment paradigms in this emerging molecular subset.

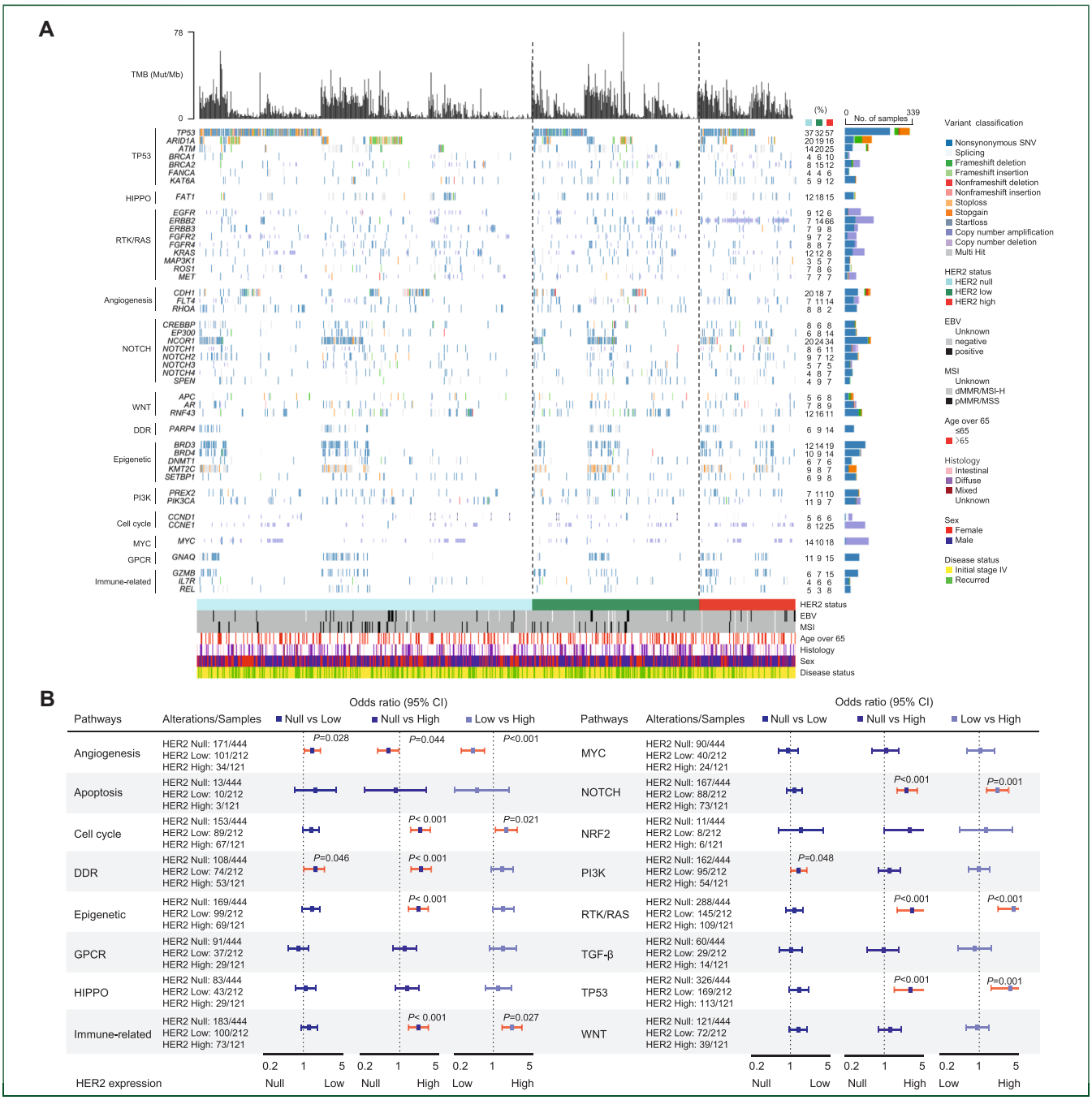


**Figure 4. Genomic landscape of patients with gastric cancer according to HER2 expression.** (A) Tumor tissue-targeted DNA sequencing results grouped by HER2 expression and related clinicopathologic features ($n = 777$). Curated pathways and selected genes altered in ≥5% of the patients are shown. Vertical dashed lines indicate groups by HER2 expression. (B) Forest plot showing the association between HER2 expression category and pathway-level genomic alterations. Numbers indicate altered samples per total samples in each HER2 group. Odds ratios (ORs) with 95% confidence intervals (CIs) represent the likelihood of harboring ≥1 genomic alteration and are shown for pairwise comparisons of HER2-null versus HER2-low, HER2-null versus HER2-high, and HER2-low versus HER2-high. The dashed line indicates OR = 1; significant associations are highlighted in red.

DDR, DNA damage response; dMMR, deficient mismatch repair; EBV, Epstein—Barr virus; HER2, human epidermal receptor 2; MSI, microsatellite instability; MSI-H, microsatellite instability—high; MSS, microsatellite stable; SNV, single nucleotide variant; pMMR, proficient mismatch repair; TMB, tumor mutation burden.

Despite its strengths, this study has several limitations. The retrospective, single-center design introduces potential selection bias and limits external generalizability. Nevertheless, we believe our dataset represents one of the largest cohorts of patients with HER2-low G/GEJ cancer with comprehensive epidemiologic, survival, and NGS data. To minimize bias in the survival analysis, we included only patients who received fluoropyrimidine and platinum-based doublet chemotherapy as first-line palliative treatment. Molecular profiling was conducted using targeted sequencing panels, which may not capture the full genomic complexity compared with whole-exome or transcriptomic approaches. In addition, pathway-level analyses were exploratory in nature, and multiple comparison adjustments were not applied. Lastly, this study was not designed to assess predictive value of HER2 status or molecular

alterations for specific therapies and thus cannot establish causality between biomarker presence and treatment response.

### *Conclusion*

HER2-low G/GEJ cancer represents a distinct molecular entity with intermediate clinicopathologic characteristics, unique genomic features, and potential therapeutic vulnerabilities, supporting its classification as a separate molecular subset. The enhanced benefit observed with ICI combination therapy suggests potential immunological vulnerability in these tumors. Further investigation, including ongoing trials assessing combinations of novel HER2-targeted therapies and angiogenesis inhibitors, is essential to develop tailored therapeutic strategies and improve patient outcomes in HER2-low G/GEJ cancer.

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## DISCLOSURE

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## DATA TRANSPARENCY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy restrictions but are available from the corresponding author on reasonable request.

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