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Clinicopathological Characteristics and Prognostic Significance of High, Low, and Absent HER2 Expression Status in Colorectal Cancer

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Abstract

Positive outcomes with HER2-directed ADCs in tumors showing low HER2 expression have challenged the traditional binary (positive/negative) categorization of HER2 status. However, the clinicopathological features and prognostic implications of varying HER2 expression levels in colorectal cancer remain incompletely understood. We conducted a multicenter retrospective analysis of patients with histologically confirmed colorectal cancer who underwent curative resection and had available HER2 status. Due to similar clinicopathological profiles, IHC 1+ and IHC 2+ cases were grouped as HER2-low. Relative to the HER2-high group, both HER2-zero and HER2-low tumors displayed significantly lower rates of perineural invasion (14.3% and 13.1% vs. 31.6%; P = 0.001 and P < 0.001), reduced proportion of stage III disease (41.8% and 39.9% vs. 56.1%; P = 0.044 and P = 0.022), higher prevalence of RAS/BRAF mutations (52.1% and 49.9% vs. 19.5%; P < 0.001 for both), and superior disease-free survival (3-year DFS rates: 78.7% and 82.4% vs. 59.3%; P < 0.001 for both). Multivariate analysis and propensity score matching further established HER2-high expression as an independent adverse prognostic factor for DFS. In summary, HER2-low colorectal tumors share characteristics with HER2-zero tumors and differ markedly from HER2-high tumors. Routine HER2 immunohistochemistry is warranted in early-stage colorectal cancer.

Keywords: ERBB2, Immunohistochemistry, Colorectal neoplasms, Clinicopathological feature

Introduction

HER2 (Erb-B2 receptor tyrosine kinase), encoded by a proto-oncogene on chromosome 17q21, contributes to oncogenesis and tumor progression [1, 2]. It is a validated therapeutic target in breast, gastric, and colorectal cancers [3-6]. While HER2 testing is currently recommended only for metastatic colorectal cancer, where targeted therapies are approved, it is not routinely performed in early-stage disease [7].

The prognostic significance of HER2 expression in colorectal cancer, particularly in early stages, remains debated. Multiple investigations have reported no clear link between HER2 protein levels and clinical outcomes [8-12]. Notably, these earlier studies

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classified HER2 positivity using breast cancer criteria that grouped IHC 2+ and 3+ as positive, which differs from current NCCN recommendations [7]. Conversely, other reports have associated HER2 overexpression with poorer survival. For instance, Park *et al*. [13] observed reduced 3-year (70.8% vs. 83.7%) and 5-year (55.1% vs. 78.3%) survival rates in HER2-overexpressing cases, with multivariate analysis confirming an independent prognostic impact. However, that study was limited by its small sample size (n = 137) and reliance on breast cancer-derived scoring criteria [13]. Similarly, Huang *et al*. [14] reported that HER2 positivity was associated with inferior survival in stage III disease, though issues with antibody selection and diagnostic standards inconsistent with NCCN guidelines weakened the findings. Overall, evidence regarding the prognostic role of HER2 in colorectal cancer continues to be inconsistent.

Moreover, HER2-targeted agents—including monoclonal antibodies (trastuzumab, pertuzumab) and tyrosine kinase inhibitors (lapatinib, neratinib, tucatinib)—have shown efficacy primarily in tumors with HER2 overexpression or amplification (IHC 3+ or IHC 2+/ISH-positive) [3-6]. Consequently, clinical attention has centered on identifying high HER2 expression. The emergence of HER2-directed ADCs, such as trastuzumab deruxtecan (T-DXd; formerly DS-8201a), has produced promising responses even in some HER2-negative tumors [15, 16]. This development renders the conventional positive/negative dichotomy inadequate for modern research and therapy. Breast cancer has accordingly adopted a refined classification: HER2-high (IHC 3+ or IHC 2+/ISH-positive), HER2-low (IHC 1+ or IHC 2+/ISH-negative), and HER2-zero (IHC 0) [17]. Motivated by this shift, we examined the pathological characteristics and clinical outcomes associated with HER2-zero, HER2-low, and HER2-high expression in a large cohort of 2783 patients with stage I–III colorectal cancer across multiple centers.

Materials and Methods

Study population

This multicenter retrospective study enrolled patients with histologically verified colorectal cancer who had HER2 status determined and underwent radical resection between April 2013 and April 2022 at The Sixth Affiliated Hospital of Sun Yat-Sen University, The First Affiliated Hospital of Shantou University Medical College, Hunan Cancer Hospital, and The Third Affiliated Hospital of Xinxiang Medical University. Exclusion criteria included middle or lower rectal tumors (< 10 cm from the anal verge), stage IV disease, non-carcinoma histology, and non-curative (R1/R2) resection. Clinicopathological data, molecular profiles, and survival information were retrieved from medical records. The study protocol received approval from the Institutional Review Board of The Sixth Affiliated Hospital of Sun Yat-Sen University.

Analysis of HER2 protein expression by IHC

HER2 immunohistochemistry was performed on formalin-fixed paraffin-embedded (FFPE) tumor tissues. Manual staining used the HercepTest antibody (Dako A/S, Glostrup, Denmark), while automated staining employed the VENTANA 4B5 antibody on the BenchMark Ultra system. Scoring followed a 0-3+ scale per standard guidelines, with 3+ indicating strong expression, 2+ equivocal, 1+ faint/barely perceptible, and 0 indicating no expression, in accordance with HERACLES diagnostic criteria [18]. Because anti-HER2 therapies are approved only for metastatic disease, reflex FISH testing was not routinely conducted in this early-stage population.

Mismatch Repair (MMR) status determination

IHC was performed to assess MMR status of MLH1, MSH2, MSH6, and PMS2 proteins. Tumors showing loss of one or more proteins were classified as deficient MMR (dMMR); intact expression of all four defined proficient MMR (pMMR) [19].

Gene mutation detection

Genomic DNA was extracted from FFPE samples using the EZgene Tissue gDNA miniprep kit. Bidirectional Sanger sequencing on an ABI Prism 3500 DX Genetic Analyzer evaluated mutations in KRAS (exons 2, 3, 4), NRAS (exons 2, 3, 4), BRAF (exon 15, V600E), and PIK3CA (exons 9 and 20).

Treatment and follow-up

All patients received radical surgery. Most stage III and high-risk stage II patients received 6 months of adjuvant chemotherapy. Surveillance consisted of

clinical exams, serum CEA measurement, and thoraco-abdomino-pelvic CT scans every 3–6 months for the first 3 years, then every 6 months for the subsequent 2 years. Follow-up data were current as of August 2022.

Propensity score matching

Propensity score matching was applied to balance baseline covariates between HER2-high and HER2-zero/HER2-low groups. A multivariable logistic regression model incorporated prognostic factors: age (< 60 vs. ≥ 60 years), rectal vs. colon location, bowel obstruction at presentation, tumor differentiation (poor vs. well/moderate), pT stage (T4 vs. T1–3), vascular/lymphatic invasion, perineural invasion, MMR status, lymph node metastasis, tumor deposits, and lymph node yield (< 12 vs. ≥ 12). Matching was performed at a 1:4 ratio (HER2-high vs. HER2-zero or HER2-low) and 1:1 (HER2-low vs. HER2-zero) using a greedy nearest-neighbor algorithm. Balance was evaluated using standardized mean differences; SMD < 0.1 indicated adequate matching [20].

Statistical analysis

Categorical variables were compared using chi-square or Fisher's exact tests. Disease-free survival (DFS) was defined as the interval from surgery to local/regional recurrence, distant metastasis, second primary malignancy, or death from any cause. Factors significant at $P < 0.05$ in univariate analysis entered multivariate models. Analyses were conducted in R version 4.0.3, with statistical significance set at $P < 0.05$.

Results and Discussion

Patient characteristics

The study identified 2768 patients with available HER2 IHC results **(Figure 1)**. Median age was 60 years (range 17–92), and 59.4% were male. According to the eighth edition TNM staging, there were 231 (8.3%) stage I, 1391 (50.3%) stage II, and 1146 (41.4%) stage III cases. Complete KRAS, NRAS, and BRAF mutation data were available for 1729 patients.

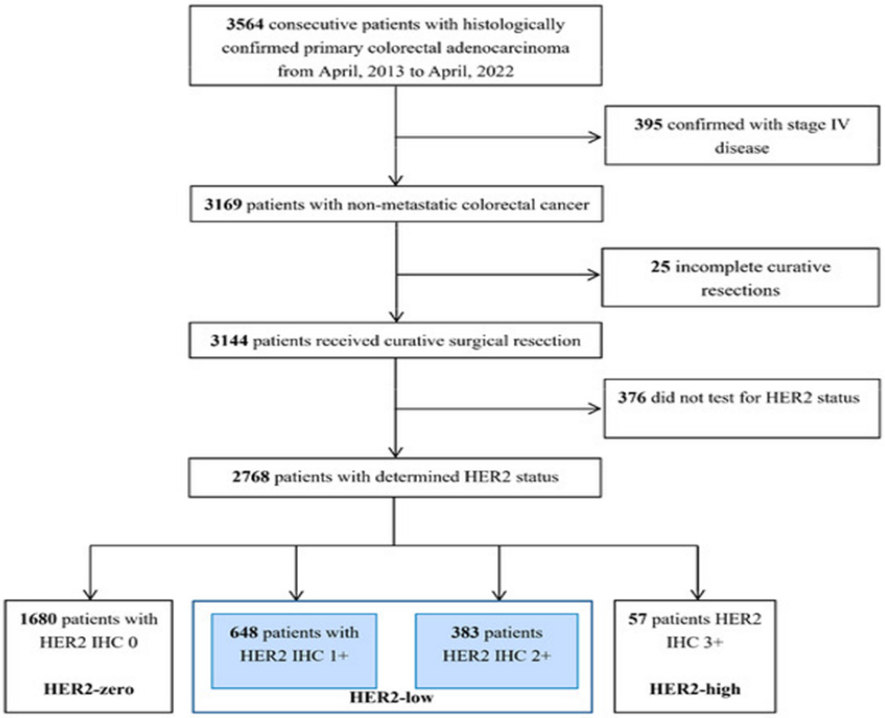


Figure 1. Flowchart illustrating the study design and patient selection process.

Distribution of HER2 expression scores (0, 1, 2, and 3)

The entire patient population was stratified by HER2 immunohistochemistry (IHC) score. The distribution

across the different staining intensities was as follows: 1680 cases with score 0, 648 with score 1, 383 with score 2, and 57 with score 3. Examples of representative HER2 IHC staining for scores 0, 1, 2, and 3 are displayed in **Figures 2a–2d**.

Across the groups, statistically significant differences were observed in tumor differentiation grade, presence of initial bowel obstruction, vascular and/or lymphatic invasion, perineural invasion, pathological T stage, and RAS/BRAF mutation status. In contrast, no notable differences were found regarding patient age, sex, tumor location, lymph node metastasis, or MMR status.

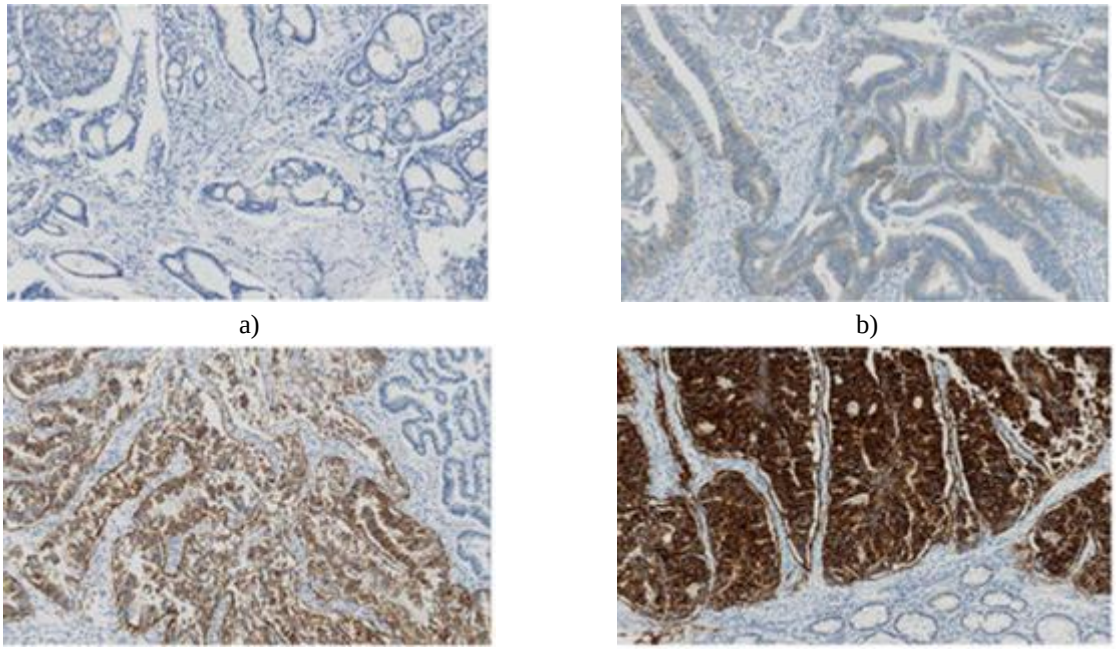


Figure 2. Typical microscopic views showing HER2 protein expression by immunohistochemistry. Panels display representative staining patterns for HER2 IHC scores 0 (a), 1 (b), 2 (c), and 3 (d) at 10× magnification.

Unique clinicopathological profiles of HER2-zero, HER2-low, and HER2-high colorectal tumors

To thoroughly investigate overlaps and distinctions in tumor features by HER2 status, we began by directly comparing the IHC 1+ and IHC 2+ categories. These two subgroups exhibited no meaningful differences across any baseline clinical or pathological variables examined (all comparison p-values exceeded 0.3).

Consequently, we grouped the cases into three categories: tumors with IHC score 0 were labeled HER2-zero, those with IHC scores 1+ or 2+ were combined as HER2-low, and IHC score 3+ tumors were designated HER2-high.

Relative to the HER2-high subset, both HER2-zero and HER2-low tumors demonstrated substantially reduced perineural invasion (14.3% compared with 31.6%, P = 0.001; and 13.1% compared with 31.6%, P < 0.001) along with a lower incidence of stage III disease (41.8% compared with 56.1%, P = 0.044; and 39.9% compared with 56.1%, P = 0.022) **(Table 1; Figures 3a and 3b)**.

Further comparison between the HER2-zero and HER2-low categories confirmed close similarity in perineural invasion rates (14.3% vs. 13.1%, P = 0.415) and in the proportion of stage III cases (41.8% vs. 39.9%, P = 0.328) **(Figures 3a and 3b)**. However, these groups diverged in several other important aspects, including the occurrence of bowel obstruction at diagnosis, degree of tumor differentiation, depth of invasion (T stage), and presence of vascular or lymphatic vessel involvement **(Table 1)**.

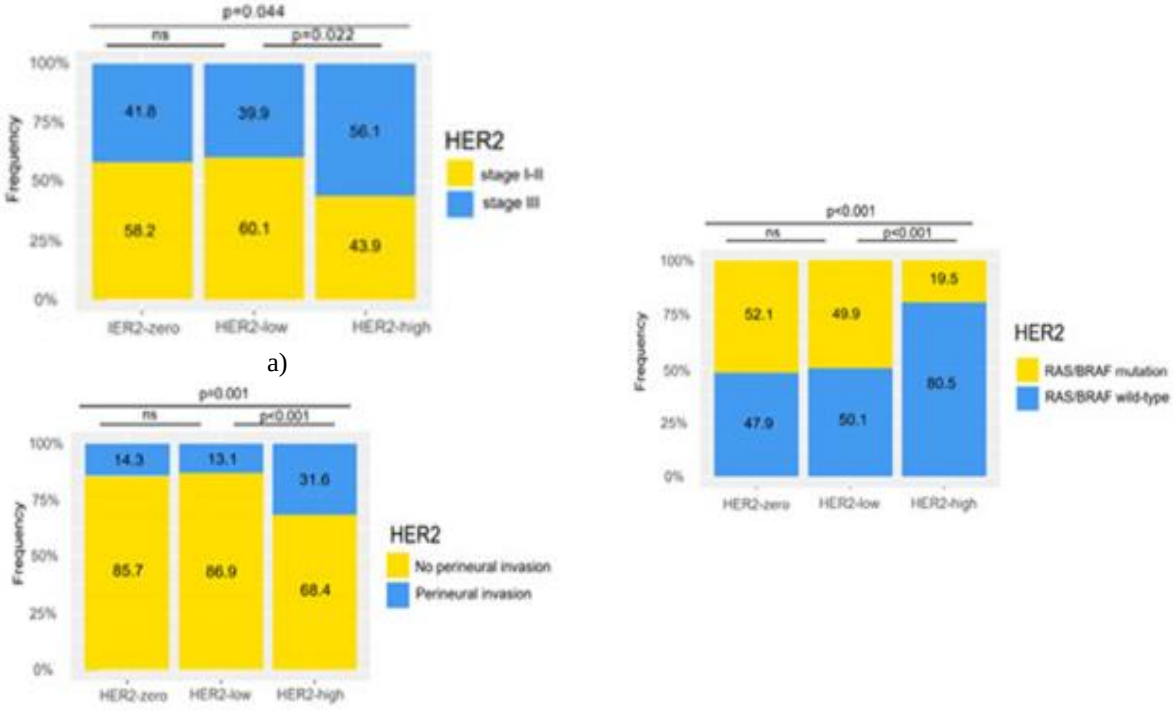


Figure 3. Variations in key clinical and pathological characteristics across HER2 expression categories, specifically (a) pathological tumor stage, (b) perineural invasion, and (c) RAS/BRAF mutation status.

Table 1. Baseline clinicopathological characteristics of the overall cohort.

Characteristics	All the population, n = 2768	HER2 High group, n = 57	HER2-Low Group, n = 1031	HER2-Zero group, n = 1680	P
	No. (%)	No. (%)	No. (%)	No. (%)	
Age, years					
< 60	1325 (47.9%)	31 (54.4%)	490 (47.5%)	804 (47.9%)	ns
≥ 60	1443 (52.1%)	26 (45.6%)	541 (52.5%)	876 (52.1%)	
Gender					
Female	1125 (40.6%)	24 (42.1%)	406 (39.4%)	695 (41.4%)	ns
Male	1643 (59.4%)	33 (57.9%)	625 (60.6%)	985 (58.6%)	
Grade of differentiation					zero vs. low, P < 0.001
Well- or moderately	2373 (85.7%)	53 (93.0%)	925 (89.7%)	1395 (83.0%)	zero vs. high, ns
Poorly	395 (14.3%)	4 (7.0%)	106 (10.3%)	285 (17.0%)	low vs. high, ns
Primary tumor site					
Left (splenic flexure, descending colon, sigmoid colon, and rectum)	1685 (60.9%)	37 (64.9%)	638 (61.9%)	1010 (60.1%)	ns
Right (cecum, ascending colon, hepatic flexure, and transverse colon)	1083 (39.1%)	20 (35.1%)	393 (38.1%)	670 (39.9%)	
Rectal cancer					
No	2719 (98.2%)	55 (96.5%)	1012 (98.2%)	1652 (98.3%)	ns
Yes	49 (1.8%)	2 (3.5%)	19 (1.8%)	28 (1.7%)	

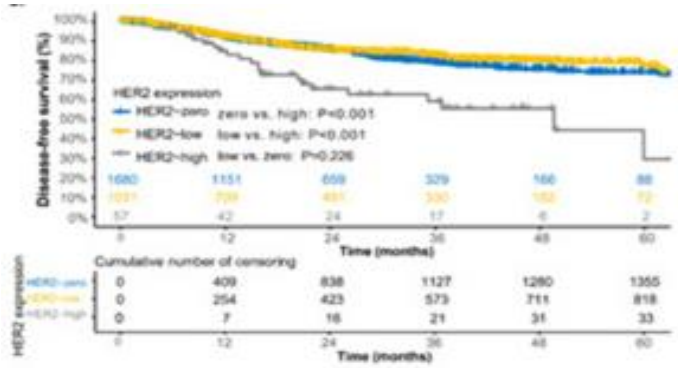
Initial bowel obstruction					zero vs. low, P < 0.001
No	2631 (95.1%)	53 (93.0%)	1000 (97.0%)	1578 (93.9%)	zero vs. high, ns
Yes	137 (4.9%)	4 (7.0%)	31 (3.0%)	102 (6.1%)	low vs. high, ns
Vascular invasion and/or lymphatic infiltration					zero vs. low, P = 0.016
No	2471 (89.3%)	47 (82.5%)	941 (91.3%)	1483 (88.3%)	zero vs. high, ns
Yes	297 (10.7%)	10 (17.5%)	90 (8.7%)	197 (11.7%)	low vs. high, P = 0.045
Perineural invasion					zero vs. low, ns
No	2375 (85.8%)	39 (68.4%)	896 (86.9%)	1440 (85.7%)	zero vs. high, P = 0.001
Yes	393 (14.2%)	18 (31.6%)	135 (13.1%)	240 (14.3%)	low vs. high, P < 0.001
No. of lymph nodes excised					
<12	284 (10.3%)	6 (10.5%)	118 (11.4%)	160 (9.5%)	ns
≥12	2484 (89.7%)	51 (89.5%)	913 (88.6%)	1520 (90.5%)	
Pathologic T stage					zero vs. low, P < 0.001
T1–T3	2349 (84.9%)	47 (82.5%)	835 (81.0%)	1467 (87.3%)	zero vs. high, ns
T4	419 (15.1%)	10 (17.5%)	196 (19.0%)	213 (12.7%)	low vs. high, ns
Lymph node metastasis					zero vs. low, ns
No	1770 (63.9%)	28 (49.1%)	678 (65.8%)	1064 (63.3%)	zero vs. high, P = 0.041
Yes	998 (36.1%)	29 (50.9%)	353 (34.2%)	616 (36.7%)	low vs. high, P = 0.016
Tumor deposit					zero vs. low, ns
No	2283 (82.5%)	41 (71.9%)	848 (82.3%)	1394 (83.0%)	zero vs. high, P = 0.047
Yes	485 (17.5%)	16 (28.1%)	183 (17.7%)	286 (17.0%)	low vs. high, ns
Pathologic N stage					zero vs. low, ns
N0	1622 (58.6%)	25 (43.9%)	620 (60.1%)	977 (58.2%)	zero vs. high, P = 0.044
N1–2	1146 (41.4%)	32 (56.1%)	411 (39.9%)	703 (41.8%)	low vs. high, P = 0.022
Mismatch repair status					
Proficient	2429 (87.8%)	55 (96.5%)	902 (87.5%)	1472 (87.6%)	ns
Deficient	339 (12.2%)	2 (3.5%)	129 (12.5%)	208 (12.4%)	
RAS/BRAF mutation					zero vs. low, ns
No	854 (49.4%)	33 (80.5%)	293 (50.1%)	528 (47.9%)	zero vs. high, P < 0.001

Yes	875 (50.6%)	8 (19.5%)	292 (49.9%)	575 (52.1%)	low vs. high, P < 0.001
Missing values					
Neoadjuvant therapy					
No	2765 (99.9%)	57 (100%)	1031 (100%)	1677 (99.8%)	ns
Yes	3 (0.1%)	0 (0%)	0 (0%)	3 (0.2%)	
Adjuvant therapy					
No	1377 (49.7%)	23 (40.4%)	526 (51.0%)	828 (49.3%)	ns
Yes	1391 (50.3%)	34 (59.6%)	505 (49.0%)	852 (50.7%)	

In addition, RAS/BRAF mutations occurred far less frequently in the HER2-high tumors than in the HER2-zero tumors (19.5% versus 52.1%, P < 0.001) and the HER2-low tumors (19.5% versus 49.9%, P < 0.001). Mutation prevalence was statistically comparable between the HER2-zero and HER2-low categories (52.1% versus 49.9%, P = 0.415) **(Figure 3c)**. Overall, these results indicate clear differences in key clinical and molecular characteristics among the HER2-zero, HER2-low, and HER2-high colorectal cancer subsets.

Prognostic impact of HER2 expression levels across the full study population and its subgroups

By the end of the observation period, the median follow-up time reached 22.4 months for all participants. The estimated 3-year disease-free survival rates stood at 59.3% (95% confidence interval 45.7–72.9) in the HER2-high group, 82.4% (95% CI: 80.0–84.8) in the HER2-low group, and 78.7% (95% CI: 76.7–80.7) in the HER2-zero group. Survival without disease recurrence was significantly worse for patients with HER2-high tumors relative to both the HER2-zero (P < 0.001) and HER2-low (P < 0.001) groups. As anticipated, disease-free survival curves showed no meaningful separation between the HER2-zero and HER2-low groups (P = 0.226) **(Figure 4a)**.



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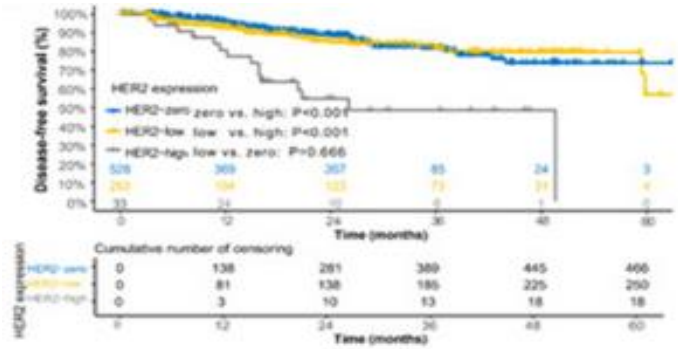

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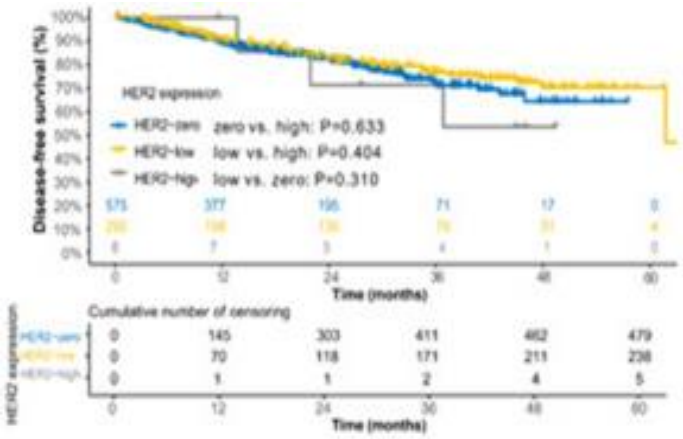

c)

Figure 4. Kaplan-Meier curves of disease-free survival by HER2 expression status. Analyses were performed in (a) the entire study population, (b) the RAS/BRAF wild-type subpopulation, and (c) the RAS/BRAF mutated subpopulation. P-values were calculated using the Cox proportional hazards model.

Similar survival patterns emerged when the analysis was restricted to patients with RAS/BRAF wild-type tumors. Individuals with HER2-low expression achieved markedly better 3-year disease-free survival (82.1% [95% CI, 77.5–86.7] vs. 48.7% [95% CI, 30.1–67.3], P < 0.001), as did those with HER2-zero status (81.7% [95% CI, 78.3–85.1] vs. 48.7% [95% CI, 30.1–67.3], P < 0.001), when compared against the HER2-high expression group **(Figure 4b)**. In contrast, no substantial differences in disease-free survival were apparent among the HER2-zero, HER2-low, and HER2-high categories within the RAS/BRAF mutated subgroup **(Figure 4c)**. Multivariate analysis identified HER2-high expression as an independent predictor of poorer disease-free survival (hazard ratio 2.05; 95% CI ,1.33–3.16; P = 0.001) **(Table 2)**. Beyond HER2 status, other independent prognostic factors included patient age, presence of rectal cancer, initial bowel obstruction, tumor differentiation grade, T stage, vascular and/or lymphatic invasion, perineural invasion, lymph node involvement, and mismatch repair status **(Table 2)**.

Table 2. Univariable and multivariable Cox regression analyses evaluating factors associated with disease-free survival in the entire study population.

Variable	No. events	No. patients	Univariate analysis		Multivariate analysis	
			HR (95% CI)	P	HR (95% CI)	P
Total	405	2768				
Age, years						
< 60	188	1325	1	0.02	1	0.033
≥ 60	217	1443	1.26 (1.04–1.53)		1.24 (1.02–1.52)	
Sex						
Female	166	1125	1	0.734		
Male	239	1643	1.03 (0.85–1.26)			
Histological differentiation						
Well or moderately differentiated	333	2373	1	0.017	1	0.041
Poorly differentiated	72	395	1.36 (1.06–1.76)		1.33 (1.01–1.73)	
Pathological T category						
T1–T3	289	2349	1	< 0.001	1	< 0.001
T4	116	419	2.27 (1.83–2.82)		1.90 (1.52–2.37)	
Vascular invasion and/or lymphatic infiltration						
No	331	2471	1	< 0.001	1	0.022
Yes	74	297	2.04 (1.59–2.63)		1.38 (1.05–1.81)	
Perineural invasion						
No	286	2375	1	< 0.001	1	

Yes	119	393	2.80 (2.26–3.46)		1.98 (1.58–2.50)	< 0.001
Number of lymph nodes removed						
≥12	57	284	1	0.007	1	0.121
<12	348	2484	1.47 (1.11–1.95)		1.26 (0.94–1.67)	
Preoperative bowel obstruction						
No	368	2631	1		1	< 0.001
Yes	37	137	2.77 (1.97–3.89)	< 0.001	2.04 (1.44–2.89)	
Lymph node involvement						
No	183	1770	1	< 0.001	1	0.005
Yes	222	998	1.87 (1.54–2.28)		1.36 (1.10–1.68)	
Tumor deposits						
No	283	2283	1	< 0.001	1	0.058
Yes	122	485	1.83 (1.48–2.27)		1.25 (0.99–1.57)	
Primary tumor location						
Left (splenic flexure, descending colon, sigmoid colon, and rectum)	267	1685	1	0.063		
Right (cecum, ascending colon, hepatic flexure, and transverse colon)	138	1083	0.82 (0.67–1.01)			
Rectal involvement						
No	390	2719	1	0.016	1	0.031
Yes	15	49	1.88 (1.12–3.15)		1.78 (1.06–3.01)	
Mismatch repair phenotype						
Proficient	384	2429	1	< 0.001	1	0.005
Deficient	21	339	0.39 (0.25–0.60)		0.52 (0.33–0.83)	
Adjuvant treatment						
No	141	1377	1	< 0.001		
Yes	264	1391	1.54 (1.25–1.89)			
HER2 expression status						
HER2-zero or HER2-low	383	2711	1	< 0.001	1	0.001
HER2-high	22	57	2.58 (1.68–3.96)		2.05 (1.33–3.16)	

Abbreviations: CI= confidence interval; HR= hazard ratio.

Analysis using propensity score matching

A 1:4 propensity score-matching procedure successfully paired all 57 HER2-high patients with individuals from the HER2-zero and HER2-low categories. Post-matching evaluation confirmed excellent balance, with standardized differences for most baseline variables remaining below 0.1.

Following this adjustment, patients in the HER2-high group displayed notably shorter disease-free survival than those in the HER2-zero group (3-year DFS rate: 59.3% [95% CI, 45.7–72.9] versus 71.6 % [95% CI, 65.5–77.7], P = 0.040) and the HER2-low group (3-year DFS rate: 59.3% [95% CI, 45.7–72.9] versus 79.3% [95% CI, 73.8–84.8], P = 0.002).

Subsequent multivariable Cox regression in the matched sets continued to identify HER2-high status as an independent indicator of increased risk for disease recurrence when benchmarked against the HER2-zero cohort (hazard ratio 1.72; 95% CI, 1.04–2.84; P = 0.035) and the HER2-low cohort (hazard ratio 2.30; 95% CI, 1.36–3.91; P = 0.002).

A separate 1:1 propensity score-matched analysis was then carried out, pairing 1032 HER2-low patients with HER2-zero patients. In this comparison, HER2-low expression did not qualify as an independent prognostic variable relative to HER2-zero tumors (hazard ratio 0.84; 95% CI, 0.67–1.06; P = 0.14) **(Table 3)**.

Table 3. Multivariable Cox proportional hazards regression analysis of disease-free survival among propensity score-matched cohorts according to HER2 expression categories.

Variable	**HER2-Zero vs. HER2-High**		**HER2-Low vs. HER2-High**		**HER2-Zero vs. HER2-Low**	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Total						
Age, years						

< 60	1	0.045	1	0.191	1	0.126
≥ 60	1.62 (1.01–2.61)		1.41 (0.84–2.37)		1.20 (0.95–1.51)	
Rectal cancer						
No	1	0.047	1	0.526	1	0.055
Yes	2.62 (1.01–6.76)		1.40 (0.49–4.00)		1.86 (0.99–3.52)	
Initial bowel obstruction						
No	1	0.434	1	0.007	1	< 0.001
Yes	1.40 (0.60–3.23)		2.71 (1.31–5.58)		2.46 (1.53–3.96)	
Histological differentiation						
Well or moderately differentiated	1	0.684	1	0.933	1	0.509
Poorly differentiated	0.79 (0.26–2.40)		1.04 (0.39–2.78)		1.14 (0.78–1.66)	
Pathological T stage						
T1–T3	1	0.010	1	0.122	1	< 0.001
T4	2.11 (1.19–3.73)		1.78 (0.86–3.69)		1.81 (1.40–2.33)	
Vascular invasion and/or lymphatic infiltration						
No	1	0.008	1	0.989	1	0.290
Yes	2.27 (1.24–4.15)		1.01 (0.46–2.18)		1.21 (0.85–1.72)	
Perineural invasion						
No	1	0.002	1	0.991	1	< 0.001
Yes	2.24 (1.34–3.74)		1.00 (0.57–1.74)		2.26 (1.73–2.95)	
Lymph node metastasis						
No	1	0.631	1	0.228	1	0.024
Yes	1.14 (0.67–1.95)		1.43 (0.80–2.54)		1.32 (1.04–1.69)	
Tumor deposits						
No	1	0.682	1	0.188	1	0.056
Yes	0.89 (0.52–1.54)		1.47 (0.83–2.61)		1.30 (0.99–1.70)	
Number of lymph nodes removed						
≥ 12	1	0.736	1	0.988	1	0.141
< 12	1.14 (0.53–2.44)		0.99 (0.47–2.09)		1.27 (0.92–1.75)	
Mismatch repair status						
Proficient	1	0.277	1	0.996	1	0.037
Deficient	0.32 (0.04–2.48)		0 (0–Inf)		0.58 (0.35–0.97)	
HER2 expression status						
Zero	1	0.035			1	0.140
Low			1	0.002	0.84 (0.67–1.06)	
High	1.72 (1.04–2.84)		2.30 (1.36–3.91)			

The primary objective of this multicenter investigation was to examine the clinicopathological characteristics and prognostic implications of varying HER2 expression levels in colorectal cancer. We analyzed data from 2768 patients across multiple institutions. The key observation was that tumors classified as HER2-low exhibited biological behavior and clinical outcomes highly similar to those of the HER2-zero group. By contrast, HER2-high tumors displayed distinctly different features and significantly worse prognosis compared with both HER2-zero and HER2-low cases. These findings support the routine use of HER2 immunohistochemistry in early-stage colorectal cancer to inform prognosis and therapeutic decision-making.

Unlike several prior studies evaluating the prognostic role of HER2 in colorectal cancer, our work adhered to the diagnostic criteria outlined in NCCN guidelines and involved a large, multicenter cohort with reduced inter-patient heterogeneity. Earlier reports noted that HER2 overexpression is more common in RAS/BRAF wild-type tumors [6, 21, 22]. Accordingly, NCCN guidelines do not recommend HER2 testing in cases with known RAS/BRAF mutations, as these patients are generally not suitable candidates for dual HER2-targeted therapy [7]. In the present study, we further assessed the prognostic impact of HER2 status stratified by RAS/BRAF mutation profile. To the best of our knowledge, this is the first comprehensive evaluation of clinicopathological features, molecular alterations, and prognostic significance in HER2-zero and HER2-low early-stage colorectal cancer.

Compared with the other groups, HER2-high tumors were more frequently associated with perineural invasion and advanced TNM stage, while showing a

markedly lower rate of RAS/BRAF mutations. No significant difference in mutation frequency existed between HER2-low and HER2-zero tumors. Although some minor clinicopathological variations persisted between HER2-low and HER2-zero cases, their long-term outcomes were comparable. Thus, HER2-low tumors align closely with HER2-zero tumors and differ substantially from HER2-high tumors. Our results align with recent data from metastatic colorectal cancer, where HER2-low cases demonstrated similar prognostic behavior and molecular profiles to HER2-zero tumors [23]. This pattern contrasts with observations in breast cancer, where HER2-low tumors possess unique biological traits, distinct molecular features, and different clinical outcomes relative to HER2-zero tumors [24]. In breast cancer, HER2-low disease is typically linked to higher hormone receptor positivity, increased PIK3CA mutations, reduced TP53 mutations, and altered pathologic complete response rates after neoadjuvant therapy [25].

In our cohort, the adverse prognostic effect of HER2-high expression in the RAS/BRAF wild-type population was similar to that observed in the overall cohort. However, within the RAS/BRAF-mutated subgroup, no significant survival differences were observed across HER2 categories. RAS/BRAF mutations are highly prevalent in colorectal cancer and play a critical role in tumor development and progression [26]. HER2 amplification accounts for approximately 5% of metastatic colorectal cancer cases [27]. Our findings suggest that the oncogenic drive of HER2 may vary depending on RAS/BRAF status, a major downstream signaling pathway of HER2 [26]. It is well established that HER2 overexpression can exhibit significant intra- and inter-tumoral heterogeneity [28]. The therapeutic efficacy of trastuzumab also differs across breast, gastric, and colorectal cancers despite HER2 overexpression or amplification [4, 29, 30]. While the DESTINY-Breast04 trial demonstrated substantial improvements in progression-free and overall survival with T-DXd in HER2-low advanced breast cancer [31], response rates to DS-8201 in HER2-low colorectal cancer were limited according to the DESTINY-CRC01 study [32]. This reduced efficacy may stem from activation of RAS/RAF signaling in HER2-low metastatic colorectal tumors, which show enrichment of RAS-related pathways [23, 27]. Intratumoral heterogeneity may provide another explanation, as one study reported that only about 20% (up to 60%) of cells express HER2 in HER2-low colorectal cancers [23].

Several limitations should be acknowledged. As with any retrospective study, incomplete records and missing data are inherent drawbacks. Additionally, FISH testing was not performed, so some IHC 2+ cases were included in the HER2-low group without confirmation of HER2 amplification. However, this is unlikely to undermine our conclusions. Any HER2 2+/FISH+ tumors misclassified as HER2-low would be expected to have poorer outcomes, similar to those of HER2-high cases; excluding them would likely accentuate the survival difference between HER2-low and HER2-high groups. Importantly, the majority of IHC 2+ colorectal tumors are FISH-negative, with HER2 amplification occurring in only 5–11% of IHC 2+ cases [18]. Therefore, our results were minimally impacted by the absence of FISH testing. Finally, our molecular analysis was restricted to RAS/BRAF status; the lack of broader genomic and multi-omic profiling limited deeper exploration of differences among HER2 subgroups.

Conclusion

In summary, this study demonstrates that HER2-low colorectal tumors closely resemble HER2-zero tumors, whereas they differ significantly from HER2-high tumors. Both HER2-zero and HER2-low expression were linked to more favorable disease-free survival in non-metastatic colorectal cancer compared with HER2-high expression. Routine HER2 immunohistochemistry should be incorporated into the evaluation of early-stage colorectal cancer to support more precise risk stratification and treatment strategies in clinical practice.

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