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Research Article

# Pathologic Complete Response to Neoadjuvant Chemotherapy in HER2-Low Versus HER2-Zero Breast Cancer: A Retrospective Cohort Study

**Shirin Haghighat[1], Monirsadat Ghorashi[2], Fatemeh Kazemzadeh[3], Mohammadmehdi Kazemzadeh[3], Sedigheh Tahmasebi[4], Ramin Ajami[5*]**

[1]Department of Hematology and Medical Oncology, Shiraz University of Medical Sciences, Shiraz, Iran.

[2]Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

[3]Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

[4]Breast Disease Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

[5]Department of Oncology (Neuroendocrine and Gastrointestinal Malignancies), Royal Free Hospital, London, United Kingdom.

***Corresponding author:** Ajami R, Department of Oncology (Neuroendocrine and Gastrointestinal Malignancies), Royal Free Hospital, London, United Kingdom.

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**Abstract**

**Purpose:** Breast cancers with low human epidermal growth factor receptor 2 expression (HER2-low), defined as immunohistochemistry IHC 1+ or IHC 2+ with negative in situ hybridization, represent a distinct subset of HER2-negative disease. While this status is significant in metastatic breast cancer, its effect on neoadjuvant chemotherapy response and survival in early-stage disease is uncertain. This study investigates the relationship between HER2-low expression, pathological complete response (pCR), and survival outcomes in early-stage HER2-negative breast cancer. **Patients and Methods:** We conducted a retrospective analysis of 934 women with stage II–III HER2-negative breast cancer who received neoadjuvant chemotherapy from 2016 to 2021. Tumors were categorized as HER2-zero (IHC 0) or HER2-low (IHC 1+ or IHC 2+ with negative in situ hybridization). The primary endpoint was pathological complete response (pCR), defined as absence of residual invasive disease in both the breast and axillary lymph nodes (ypT0/Tis ypN0). Secondary endpoints included 5-year disease-free survival (DFS) and overall survival (OS). Data on hormone receptor status (estrogen receptor [ER] and progesterone receptor [PR]) and menopausal status were collected. We used multivariable logistic regression to identify pCR predictors, while survival outcomes were analyzed with Kaplan–Meier estimates; differences between groups were tested with the log-rank test. **Results:** About half of the tumors were HER2-low, with most patients presenting hormone receptor–positive disease. HER2-low tumors showed notably lower pCR rates compared to HER2-zero tumors. ER and PR positivity independently correlated with a decreased chance of achieving pCR in both HER2 subgroups. After a median follow-up of five years, there were no significant differences in DFS or OS between the groups. **Conclusion:** HER2-low breast cancer shows lower response rates to neoadjuvant chemotherapy, especially in hormone receptor–positive cases, yet there is no negative impact on 5-year survival..

**Keywords:** Breast Cancer; HER2-low; HER2-negative; Neoadjuvant Chemotherapy.

## Introduction

Breast cancer remains the most prevalent malignancy among women worldwide, with approximately 2.3 million new cases diagnosed annually [1-3]. Although incidence increases with age, nearly 7% of cases occur in women younger than 40 years, underscoring the biological and clinical heterogeneity of the disease across age groups [4]. Despite major advances in screening, systemic therapy, and multidisciplinary management, breast cancer continues to account for substantial morbidity and mortality globally, particularly in countries undergoing economic and demographic transition [5-7].

Contemporary breast cancer management relies on molecular profiling, especially the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). These markers are essential for current subtype classification and guiding therapy decisions [4, 8, 9]. HER2-positive tumors, characterized by HER2 protein overexpression or gene amplification (IHC 3+ or IHC 2+/ISH+), account for about 20–30% of cases and are known for their aggressive nature. Yet, prognosis has improved significantly with HER2-targeted therapies like trastuzumab and pertuzumab [10-15]. Conversely, roughly 80% of breast cancers lack HER2 amplification and are classified as HER2-negative, thus limiting the use of HER2-targeted treatments in these cases [16].

Recent refinements in HER2 assessment have led to the recognition of a HER2-low subgroup, defined as tumors with immunohistochemistry (IHC) 1+ or IHC 2+ staining in the absence of ERBB2 gene amplification [16-22]. HER2-low tumors account for nearly half of breast cancers previously classified as HER2-negative and have gained clinical relevance following the demonstrated efficacy of antibody-drug conjugates, particularly trastuzumab deruxtecan, in metastatic disease [19]. Beyond therapeutic implications, accumulating evidence suggests that HER2-low tumors may represent a biologically distinct subset rather than a purely technical classification.

HER2-low breast cancers are consistently more likely to be hormone receptor-positive and tend to fall into luminal molecular subtypes. They also show lower proliferative activity and reduced Ki-67 expression compared to HER2-zero tumors [16, 29, 35-37]. These biological traits are known to provide some resistance to cytotoxic chemotherapy and correlate with lower rates of pathological complete response (pCR) in neoadjuvant treatments [32, 39-41]. Consequently, HER2-low status might indicate a tumor phenotype driven mainly by hormone signaling, with decreased chemosensitivity and unique therapeutic vulnerabilities, regardless of eligibility for HER2-targeted therapies.

Neoadjuvant chemotherapy (NACT) is frequently used to treat patients with locally advanced and high-risk early-stage breast cancer, aiming to shrink tumors and assess the treatment response in vivo [22, 23]. A complete response at the pathological level after NACT is strongly associated with improved long-term outcomes, especially for aggressive subtypes like triple-negative breast cancer [24]. However, dependable markers for chemotherapy response in HER2-negative breast cancer are limited, and the role of low HER2 expression in affecting chemosensitivity remains uncertain [25].

Previous research on how HER2-low status influences the response to neoadjuvant chemotherapy and survival has produced mixed results. Some studies report notably lower pCR rates in HER2-low tumors compared to HER2-zero tumors, while others find no significant differences in response or long-term outcomes [17, 26]. Many of these analyses are hampered by small sample sizes, diverse patient groups, inconsistent HER2 testing methods, or limited subgroup analysis, which fuels ongoing uncertainty about the predictive and prognostic significance of HER2-low expression in early-stage disease [16, 20].

Against this background, the present study aimed to evaluate whether HER2-low expression, compared with HER2-zero status, is associated with pathological complete response to neoadjuvant chemotherapy in patients with stage II–III HER2-negative breast cancer. Secondary objectives included comparison of disease-free survival (DFS) and overall survival (OS) between these groups, with exploratory analyses within the HER2-low subgroup. By anchoring HER2-low expression within a biologically plausible framework of hormone receptor enrichment and reduced proliferation, this study seeks to clarify its role as a predictive construct in the neoadjuvant setting and to inform future strategies for treatment stratification in HER2-negative breast cancer.

## Methods

### Study Design and Setting

This was a retrospective cohort study utilizing clinical and pathological data from patients treated at the Breast Cancer Research Center of the Motahari Clinic between 2016 and 2021, with longitudinal follow-up for survival outcomes. This study aimed to evaluate the influence of HER2 expression on the pathological response to neoadjuvant chemotherapy and subsequent survival outcomes in patients with HER2-negative breast cancer.

### Study Population and Sample Size

The study population consisted of female patients aged 18–75 years with histologically confirmed invasive breast carcinoma who received neoadjuvant chemotherapy followed by definitive breast

surgery at the Breast Cancer Research Center of the Motahari Clinic, Shiraz, Iran, between January 2016 and December 2021. This retrospective cohort study with longitudinal follow-up was designed to evaluate pathological response and survival outcomes.

Patients were eligible if they had stage II–III HER2-negative breast cancer, defined according to contemporaneous ASCO/CAP guidelines based on immunohistochemistry (IHC) and in situ hybridization (ISH) testing [27, 28]. Tumors were classified as HER2-zero (IHC score 0) or HER2-low (IHC 1+ or IHC 2+ with negative ISH), consistent with emerging consensus definitions within the HER2-negative spectrum [16, 29, 38]. HER2 assessment was performed locally at the time of diagnosis, without centralized pathology review. Since identifying HER2-zero versus HER2-low tumors can vary between observers, especially at low expression levels, there is a potential for misclassification bias that cannot be entirely ruled out [29, 38].

Prespecified exclusion criteria were consistently applied to all potentially eligible patients. Patients were excluded if they had inflammatory breast cancer, bilateral synchronous breast cancer, or a history of invasive malignancy (except non-melanoma skin cancer or in situ cervical carcinoma) due to their unique biology, treatment strategies, and prognosis. Additionally, patients who had prior systemic therapy for breast cancer, did not complete the planned neoadjuvant chemotherapy, or did not undergo definitive breast surgery were excluded because pathological complete response could not be reliably assessed in these cases. Patients with missing, indeterminate, or unavailable HER2 or hormone receptor (ER/PR) status were also excluded from the primary analysis.

Only patients with comprehensive clinicopathologic data and available surgical pathology after neoadjuvant chemotherapy were included in the final analysis. Pathological complete response was evaluated solely in patients who underwent surgery after neoadjuvant treatment.

This study's sample size comprises all eligible patients treated during the study period with complete data, without a formal pre-study size calculation. The final count of 934 patients represents the entire source population meeting eligibility criteria within the specified timeframe, enhancing statistical power and reducing selection bias typical in retrospective cohorts.

Patients received standard neoadjuvant chemotherapy following institutional protocols and current international guidelines for early-stage and locally advanced breast cancer [22, 23]. The neoadjuvant treatment mainly involved anthracycline- and taxane-based regimens, either sequentially or combined, which is the standard approach for HER2-negative breast cancer [8, 22]. Typical regimens included doxorubicin plus cyclophosphamide followed by a taxane, or upfront taxane-based treatments, with the specific choice based on tumor features, patient performance status, and physician judgment.

Chemotherapy was administered at standard doses and schedules. Dose-dense regimens were used selectively based on clinical judgment and patient tolerance. The duration of neoadjuvant chemotherapy typically ranged from 4 to 8 cycles, depending on the regimen and clinical response. No patients received HER2-targeted therapy, consistent with HER2-negative disease status.

Treatment selection was not based on HER2-low versus HER2-zero status, since HER2-low did not affect neoadjuvant treatment choices during the study period. As a result, there was some variation in chemotherapy regimens across the cohort; however, all patients received contemporary cytotoxic chemotherapy regarded as standard care for HER2-negative breast cancer. Considering this real-world treatment setting, the type of chemotherapy regimen was not used as a stratification variable in the main analyses.

### Data Collection

Data, including demographic information (age and menopausal status), tumor characteristics (size, histological type, grade, and hormone receptor status), treatment details (neoadjuvant chemotherapy regimens and radiotherapy), and pathological outcomes, were extracted from the center's electronic and paper medical records. HER2 expression was assessed by experienced pathologists according to contemporaneous ASCO/CAP criteria.. Pathological complete response (pCR) was defined as the absence of invasive cancer in both the breast tissue and lymph nodes following neoadjuvant chemotherapy (ypT0/Tis ypN0), allowing for the presence of ductal carcinoma in situ. Radiotherapy data included chest wall, axillary, and intraoperative radiotherapy.

### Outcome Measures

Pathological complete response (pCR) was defined as the absence of residual invasive disease in the breast and axillary lymph nodes at surgery following neoadjuvant chemotherapy (ypT0/Tis ypN0).

For survival analyses, disease-free survival (DFS) and overall survival (OS) were calculated from the date of histopathologic diagnosis. DFS was defined as the time to first documented local, regional, or distant recurrence, or death from any cause. OS was defined as the time to death from any cause. Recurrence and survival events were ascertained through institutional medical records. Patients without events were censored at the date of last clinical follow-up.

## Statistical Analysis

Descriptive statistics summarized clinicopathologic features. Continuous variables were compared with independent-sample t tests or ANOVA, while categorical variables were analyzed using

chi-square or Fisher's exact tests, as appropriate.

Pathological complete response (pCR) was assessed through both univariable and multivariable logistic regression models. A priori covariates included age at diagnosis, hormone receptor status (ER/PR), menopausal status, tumor size, histologic grade, lymphovascular invasion, and HER2 status (HER2-low vs HER2-zero), based on known clinical relevance and prior literature. Chemotherapy regimen type was not included as a covariate due to heterogeneity and lack of protocolized assignment. Interaction terms between HER2 status and hormone receptor status were explored in prespecified analyses. Only complete cases were included in regression analyses.

Survival outcomes were evaluated with the Kaplan–Meier method and compared using log-rank and Wilcoxon tests. No multivariable Cox models were conducted. Subgroup analyses, such as those based on menopausal status, were planned as exploratory. Due to their exploratory purpose and the small number of main hypotheses, no formal adjustment for multiple comparisons was made; hence, subgroup results were interpreted cautiously.

Subgroup analyses were prespecified as exploratory and interpreted descriptively.

All statistical analyses were performed using IBM SPSS Statistics, version 26, with a two-sided significance threshold of $p<0.05$.

### Ethical Considerations

The study protocol was approved by the Institutional Review Board of the Motahari Clinic (Approval number IR.SUM.MED.REC.1403.600). Given the retrospective nature and anonymization of the patient data, the need for informed consent was waived. All procedures complied with the Declaration of Helsinki and local data protection regulations.

## Results

### Patient Demographics and Tumor Characteristics:

The overall cohort consisted of 934 patients with invasive breast cancer who received neoadjuvant chemotherapy. The mean age at diagnosis was 46.81 years (SD: 10.88), with a 95% confidence interval of 46.02 to 47.60 years . When stratified by HER2 status, patients with HER2 1+/2+ tumors were slightly older (mean age 47.36 years, SD: 11.01) than those with HER2-Zero tumors (mean age: 45.10 years, SD: 10.61). This age difference was not statistically significant (ANOVA, p= 0.077). The average tumor size in the entire population was 2.34 cm (SD: 1.97). Patients with HER2 1+/2+ had a slightly larger mean tumor size of 2.40 cm compared to 2.24 cm in HER2-negative patients, but this difference was not statistically significant (p=0.245). Most tumors were invasive ductal carcinomas: 81.2% in HER2-zero and 85.6% in HER2-low groups (p=0.078). Grade 2 was most common in both HER2-zero (45%) and HER2-low (46.7%) groups. HER2-low tumors exhibited higher rates of vascular and perineural invasion (p=0.006) and a greater proportion of in situ components (40.3% vs. 33.8%, p=0.045). There was no significant difference in tumor necrosis presence between the groups (p=0.573) (Table 1).

| Variable | HER2-zero (N=373) | HER2-low (N=561) | P-value |
|---|---|---|---|
| Age (years) (mean ± SD) | 45.10 ± 10.61 | 47.36 ± 11.00 | 0.077 |
| Tumor size (cm) (mean ± SD) | 2.23 ± 1.95 | 2.40 ± 1.97 | 0.245 |
| **Tumor type (%)** | | | 0.078χ2 |
| Invasive ductal carcinoma | 303 (81.2%) | 480 (85.6%) | |
| Others | 70 (18.8%) | 81 (14.4%) | |
| **Tumor grade (%)** | | | 0.534χ2 |
| Grade 1 | 43 (11.5%) | 74 (13.2%) | |
| Grade 2 | 168 (45.0%) | 262 (46.7%) | |
| Grade 3 | 64 (17.2%) | 78 (13.9%) | |
| Missing | 98 (26.3%) | 147 (26.2%) | |
| **Multifocality (%)** | | | 0.992χ2 |
| Multifocal | 353 (94.6%) | 531 (94.7%) | |
| Non-multifocal | 20 (5.4%) | 30 (5.3%) | |
| **Multicentricity (%)** | | | 0.157χ2 |
| Multicentric | 373 (100%) | 558 (99.5%) | |

| | | | |
|---|---|---|---|
| Non-multicentric | 0 (0.0%) | 3 (0.5%) | |
| **In-situ component (%)** | | | 0.045χ2 |
| Absent | 247 (66.2%) | 335 (59.7%) | |
| Present | 126 (33.8%) | 226 (40.3%) | |
| **Tumor necrosis (%)** | | | 0.573χ2 |
| Absent | 250 (67.0%) | 366 (65.2%) | |
| Present | 123 (33.0%) | 195 (34.8%) | |
| **Margin status (%)** | | | 0.513χ2 |
| Negative | 362 (97.1%) | 540 (96.3%) | |
| Positive | 11 (2.9%) | 21 (3.7%) | |
| **Tumor invasion (%)** | | | 0.006χ2 |
| None | 217 (58.2%) | 261 (46.6%) | |
| Perineural | 19 (5.1%) | 32 (5.7%) | |
| Vascular | 95 (25.5%) | 177 (31.6%) | |
| Both (PN+V) | 42 (11.3%) | 90 (16.1%) | |
| *Values are presented as mean ± standard deviation or n (%). Group comparisons were performed using independent t-tests for continuous variables and chi-square tests for categorical variables. Statistically significant p-values (<0.05) are bolded. Abbreviations: HER2, human epidermal growth factor receptor 2; SD, standard deviation; PN, perineural; V, vascular. | | | |

**Table 1:** Clinicopathological features of patients stratified by HER2-zero(Negative) and HER2-low breast cancer treated with neoadjuvant chemotherapy.

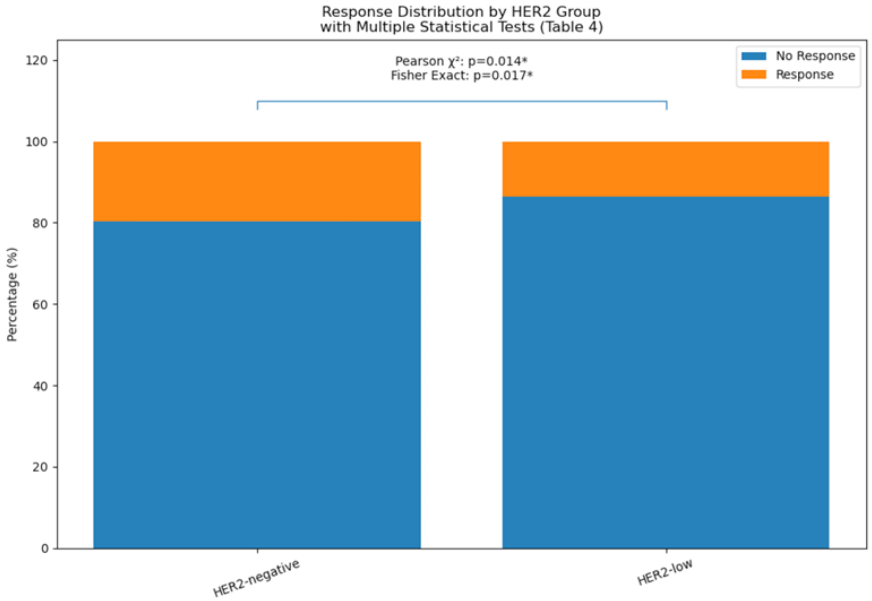



**Figure 1:** Pathologic complete response rates according to HER2 status following neoadjuvant chemotherapy.

### Histopathologic Characteristics across HER2 Groups

The distribution of tumor grades did not differ significantly between the HER2-zero and HER2-low groups ($\chi^2$=2.190, df=3, p=0.534). Grade 2 was the most common in both groups, with 45.0% in HER2-zero and 46.7% in HER2-low. Logistic regression, using grade 1 as the reference, showed no significant differences in the odds of higher grades in either group: for HER2-zero, the odds ratios for grades 2 and 3 were 0.523 (p=0.311) and 0.349 (p=0.059), respectively; for HER2-low, the odds ratios were 1.621 (p=0.539) for grade 2 and 1.939 (p=0.926) for grade 3. Overall, these results indicate no substantial difference in histologic grade distribution based on HER2 status.

A notable difference was found in invasion patterns across HER2 groups ($\chi^2$=13.309, df=3, p=0.004). The HER2-low group showed a greater percentage of vascular invasion (31.6% vs 25.5%) and dual lymphovascular and perineural invasion (16.1% vs 11.3%) compared to the HER2-zero group. Logistic regression using "no invasion" as the baseline confirmed that both vascular and dual invasion were significantly linked to HER2 expression (p=0.001 for the relevant contrasts). These findings suggest that HER2-low tumors have a higher invasive potential, especially for vascular and dual invasion.

### Pathological Complete Response (pCR)

The overall pathological complete response (pCR) rate was 16.0%. When divided by HER2 status, HER2-negative patients had a notably higher pCR of 19.6%, compared to 13.5% in the HER2 1+/2+ group (Chi-square = 6.06, p = 0.014) (Table 2). This significant difference highlights the potential clinical variation in chemotherapy effectiveness based on HER2 expression, even among those without HER2 amplification (Figure 1).

| HER2 Group | Response: No | Response: Yes | Total |
|---|---|---|---|
| Count | | | |
| HER2-negative (0) | 300 | 73 | 373 |
| HER2-low (1+/2) | 485 | 76 | 561 |
| Total Count | 785 | 149 | 934 |
| % within HER2 Group | | | |
| HER2-negative (0) | 80.40% | 19.60% | 100.00% |
| HER2-low (1+/2+) | 86.50% | 13.50% | 100.00% |
| Total | 84.00% | 16.00% | 100.00% |
| % within Response | | | |
| HER2-negative (0) | 38.20% | 49.00% | 39.90% |
| HER2-low (1+/2+) | 61.80% | 51.00% | 60.10% |
| Total | 100.00% | 100.00% | 100.00% |

*Pathologic complete response (pCR) was defined as ypT0/Tis ypN0. Group comparisons were performed using the chi-square test. Abbreviations: pCR, pathologic complete response.

**Table 2:** Crosstabulation of Response by HER2 Group: Association between HER2 status and pathologic complete response following neoadjuvant chemotherapy.

### Hormone Receptor Subgroup Analysis

Exploratory subgroup analyses were performed to assess pathological complete response (pCR) according to hormone receptor (ER/PR) status within HER2-zero and HER2-low tumors. In both HER2 groups, ER/PR-negative tumors demonstrated higher pCR rates compared with ER/PR-positive tumors (Table 3). Among ER/PR-negative tumors, pCR rates were similar between HER2-zero and HER2-low groups. Among ER/PR-positive tumors, pCR rates were numerically lower in the HER2-low group compared with the HER2-zero group; however, this difference did not reach statistical significance and should be interpreted with caution.

| HER2 Group | ER/PR Group | No pCR (n) | pCR (n) | pCR (%) | p-value |
|---|---|---|---|---|---|
| HER2-negative (0) | ER-/PR-(n = 115) | 81 | 34 | 29.6% | 0.001 |
| | ER+/PR + (n =258) | 219 | 39 | 15.1% | |
| HER2-low (1+/2) | ER-/PR-(n = 86) | 60 | 26 | 30.2% | <0.001 |
| | ER+/PR+ (n =475) | 425 | 50 | 10.5% | |
| HER2 Comparison within ER-/PR- | HER2-neg: 34/115 vs HER2-low: 26/86 | | | 29.6% vs 30.2% | 0.919 |
| HER2 Comparison within ER+/PR+ | HER2-neg: 39/258 vs HER2-low: 50/475 | | | 15.1% vs 10.5% | 0.069 |
| *P-values were derived from chi-square tests. Abbreviations: ER, estrogen receptor; PR, progesterone receptor; pCR, pathologic complete response. | | | | | |

**Table 3:** Pathologic complete response rates stratified by hormone receptor status and HER2 expression.

### Survival Outcomes

In this study, we compared disease-free survival (DFS) and overall survival (OS) between HER2-zero and HER2-low tumors over five years. The average DFS was 51.20 months (95% CI, 48.81–53.58) for HER2-zero patients and 49.66 months (95% CI, 47.76–51.57) for HER2-low patients. Kaplan–Meier analysis showed no significant difference (log-rank $\chi^2$=0.881, p=0.348). The five-year DFS rates were 74% for the HER2-zero group and 71% for the HER2-low group, with overlapping confidence intervals (69%–78% vs. 66%–75%), suggesting no clear separation of survival curves (Table 4, Figure 2).

Similarly, no statistically significant difference in OS was observed between the HER2-zero and HER2-low groups (log-rank $\chi^2$=0.147, p=0.702). Although HER2-low tumors demonstrated numerically lower survival estimates, these differences were not statistically significant. Overall, univariable Kaplan–Meier analyses showed no evidence of an association between HER2 status and DFS or OS within the 5-year observation period (Table 4).

| **DFS (Duration -Free Survival)** | | | | | |
|---|---|---|---|---|---|
| **Group** | **Mean Time (months)** | **Std. Error** | **Median** | **Lower 95% CI** | **Upper 95% CI** |
| Zero | 51.195 | 1.219 | -— | 48.806 | 53.584 |
| 1+ & 2+ | 49.664 | 0.972 | — | 47.758 | 51.570 |
| **OS (Overall Survival)** | | | | | |
| **Group** | **Mean Time (months)** | **Std. Error** | **Median** | **Lower 95% CI** | **Upper 95% CI** |
| Zero | 56.068 | 0.733 | — | 54.631 | 57.505 |
| 1+ & 2+ | 55.749 | 0.592 | — | 54.588 | 56.909 |
| *Log-Rank (Mantel-Cox): $\chi^2 = 0.881$, df = 1, p = 0.348 | | | | | |
| **Duration-free & overall Survival outcomes were estimated using the Kaplan-Meier method and compared using log-rank tests. CI indicates 95% confidence interval. Log-Rank (Mantel-Cox): $\chi^2 = 0.147$, df = 1, p = 0.702 | | | | | |

**Table 4:** Five-year disease-free survival and overall survival estimates according to HER2 status.

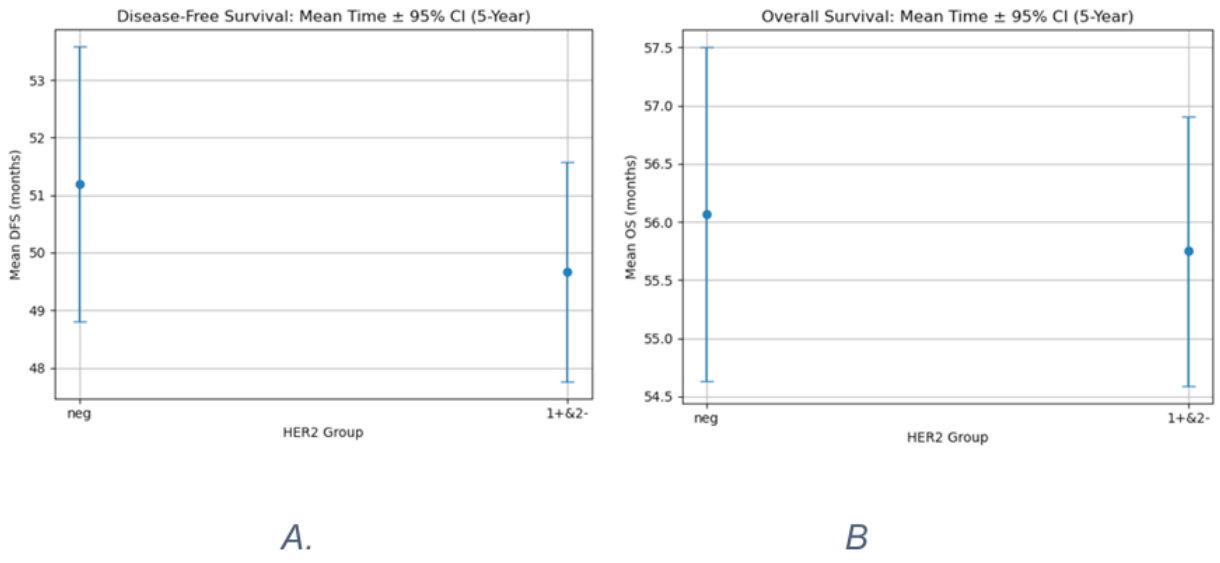



**Figure 2:** Mean survival-time estimates of (A) DFS ; (B) OS stratified by HER2 status.

### Menopausal Status and Response

Among non-menopausal breast cancer patients, a significant link was found between HER2 expression (HER2=0 vs. HER2-low) and treatment response. Chi-square analysis showed a p-value of 0.024, while Fisher's exact test confirmed this with p=0.016, suggesting HER2-low tumors may be less responsive to treatment in this group. Specifically, 60.7% of non-responders had HER2-low tumors compared to 39.3% with HER2=0, highlighting a clear response difference. Conversely, in menopausal patients, this association was not significant (chi-square p=0.288 and Fisher's exact p=0.179), indicating that HER2 status did not significantly influence treatment response in this subgroup.

### HER2 and Hormone Receptor Crosstab

Our results emphasize the important predictive role of hormone receptor status, especially ER and PR, in forecasting treatment response. This is particularly evident in the HER2-low subgroup of breast cancer patients. Logistic regression showed that ER positivity was linked to lower odds of response, with a stronger effect observed in HER2-low patients (OR=0.26; 95% CI: 0.15-0.44; p=0.001) compared to HER2=0 patients (OR=0.43; 95% CI: 0.25-0.72; p=0.002).

Similarly, PR status was associated with lower odds of treatment response, with ORs of 0.47 (95% CI: 0.28-0.79; p = 0.004) in the HER2=0 group and 0.32 (95% CI: 0.19-0.52; p = 0.001) in the HER2-low group. These findings are consistent with the lower chemosensitivity generally observed in hormone receptor-positive disease and should not be interpreted as indicating inferior long-term prognosis.

### HER2 and Menopausal Status Crosstab

This study examined the relationship between menopausal status and the likelihood of achieving a pathological complete response (pCR) across different HER2 expression groups. In the HER2-zero cohort (n = 373), 41.0% of patients were menopausal, while 59.0% were not. Among menopausal women, 17.0% attained pCR, compared to 21.4% of those who were non-menopausal. This difference was not statistically significant ($\chi^2 = 1.095$, p = 0.295).

Similarly, in the HER2-low cohort (n = 561), 44.7% of patients were menopausal and 55.3% were non-menopausal. pCR rates were comparable between menopausal (13.1%) and non-menopausal patients (13.9%), with no significant association observed ($\chi^2 = 0.062$, p = 0.803). Overall, menopausal status alone was not associated with pCR within either HER2-zero or HER2-low tumors.

Exploratory analyses were also performed to assess treatment response according to menopausal status across HER2 expression groups. Formal interaction testing between HER2 status and menopausal status was not performed; therefore, these subgroup findings should be interpreted cautiously as descriptive and hypothesis-generating.

Conversely, multivariable logistic regression analyses revealed that hormone receptor status significantly predicted treatment response. ER and PR positivity were independently linked to reduced odds of pCR in both HER2-zero and HER2-low tumors, with a notably greater decrease seen in the HER2-low subgroup.

### Discussion

Our research highlights key differences between HER2-low and HER2-zero breast cancers, especially regarding their response to neoadjuvant chemotherapy. We found notably lower rates of pathological complete response (pCR) in HER2-low tumors, aligning with emerging evidence indicating intrinsic chemoresistance in this subgroup. Similar trends have been reported by Lee et al. [30], although significance diminished after adjustment for hormone receptor status, and by a single-center Turkish study showing no difference in pCR between HER2-low and HER2-zero tumors [31], highlighting hormone receptor expression as a potential confounder. Our findings support this interpretation, as HER2-zero tumors exhibit a higher prevalence of hormone receptor negativity, which is associated with increased chemosensitivity. Luminal B tumors consistently achieve pCR in only 23–25% of cases, which is markedly lower than triple-negative or HER2-enriched subtypes [32].

Furthermore, we found a link between menopausal status and HER2 expression, with premenopausal women having HER2-low tumors showing notably worse treatment responses. This pattern was not seen in postmenopausal women. This indicates that hormonal factors might impact HER2-low tumor behavior through estrogen signaling or receptor crosstalk. Since this finding comes from a post-hoc subgroup analysis, it should be viewed as hypothesis-generating and needs validation in prospective studies, considering possible residual confounding.

Although pCR rates were lower, HER2-low status did not lead to worse disease-free survival (DFS) or overall survival (OS) at the five-year mark. This apparent dissociation aligns with a meta-analysis reporting comparable, and in some cases superior, DFS among HER2-low patients without pCR compared to HER2-zero counterparts [33]. Similarly, Ilie et al. [34] reported modest pCR differences without corresponding survival disparities, underscoring the complex relationship between chemosensitivity and long-term outcomes in HER2-low disease.

HER2-low breast cancer is closely linked to hormone receptor positivity, especially regarding estrogen receptor (ER) and progesterone receptor (PR) expression, setting it apart from HER2-zero tumors. Several studies indicate significantly higher ER and PR positivity in HER2-low tumors, with one large cohort reporting hormone receptor positivity in about 87% of cases [35]. This phenotype tends to have lower Ki-67 indices, lower histologic grades, and a higher prevalence of invasive ductal carcinoma [36, 37]. Notably, hormone receptor–positive/HER2-low tumors generally show lower pCR rates compared to hormone receptor–negative tumors, indicating greater chemoresistance [39-41]. Molecular profiling also reveals active estrogen signaling and androgen receptor expression in this subgroup, potentially affecting their therapeutic responses.

Our findings are consistent with earlier studies that emphasize the biological diversity within HER2-low breast cancers [29]. These

tumors include various intrinsic subtypes and gene expression patterns, with hormone receptor activity and proliferation playing central roles in predicting chemotherapy response. The HER2-low category has been thoroughly examined [16, 29, 38, 40], and our results reinforce its importance as a distinct and clinically relevant subgroup.

These findings carry significant clinical relevance. The reduced chemosensitivity observed in HER2-low tumors suggests that evaluating antibody–drug conjugates like trastuzumab deruxtecan in early-stage HER2-low breast cancer is worthwhile, especially considering the promising results seen in HER2-positive and metastatic HER2-low cases [42, 43]. From a research standpoint, thorough molecular profiling of HER2-low versus HER2-zero tumors is crucial to uncover the factors driving chemoresistance and to identify new therapeutic targets. Additionally, since HER2 expression can change after chemotherapy, ongoing monitoring over time is recommended, as HER2 variability may affect treatment outcomes [44]. Moreover, the differences observed based on menopausal status emphasize the need to consider hormonal environment when designing and stratifying clinical trials.

Future research combining multi-omics profiling with standardized HER2 scoring will be crucial to improve risk stratification and treatment approaches in HER2-low disease [44-48].

From a therapeutic standpoint, the lower pCR rates seen in HER2-low disease underscore the biological heterogeneity found within HER2-negative breast cancer. While new strategies like HER2-targeted antibody–drug conjugates, dynamic HER2 assessments, and biomarker-guided approaches are gaining interest, they were not examined in this study and are still considered investigational in the neoadjuvant context [43]. Therefore, our results should be viewed as hypothesis-generating and may guide the planning of future prospective research. Achieving consistent classification of HER2-low tumors is a critical methodological issue for such studies.

This study has several notable limitations. Firstly, its retrospective, single-center approach may introduce selection bias by excluding patients with incomplete neoadjuvant therapy, missing biomarker data, or atypical clinical presentations, which could affect its broader applicability. Secondly, the absence of data for important clinicopathologic factors like tumor grade and Ki-67 might have influenced baseline comparisons and the results of regression analyses, especially if the missing data were not random. Lastly, the variability in neoadjuvant chemotherapy regimens adds a potential confounding factor by indication that the analysis could not completely address.

In addition, HER2 assessment was performed locally without centralized review, raising the possibility of misclassification between HER2-zero and HER2-low tumors. Such misclassification would be expected to bias observed differences toward the null. Survival analyses were based on retrospective follow-up data without standardized ascertainment procedures, which may affect the precision and comparability of DFS and OS estimates. Finally, subgroup analyses, including those by hormone receptor and menopausal status, were exploratory and require prospective validation.

In summary, HER2-low breast cancer is a unique subset within HER2-negative disease, marked by lower pathological complete response rates, especially in hormone receptor–positive patients. However, this does not seem to negatively affect five-year survival in this group. These results should be viewed considering the study's methodological limitations and highlight the need for additional prospective research on treatment decisions based on HER2 levels and hormone receptor status.

**Authors Contribution**

**Shirin Haghighat:** Study conception and design, supervision, primary manuscript drafting.

**Monirsadat Ghorashi:** Clinical contribution, data interpretation, manuscript review.

**Fatemeh Kazemzadeh:** Data collection, literature review, manuscript drafting assistance.

**Mohammadmehdi Kazemzadeh:** Data collection, statistical analysis, manuscript assistance.

**Sedigheh Tahmasebi:** Specialty consultation, data interpretation.

**Ramin Ajami:** Study oversight, final approval, corresponding author.

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**Importance of the study**

**Key Objective**: Does HER2-low expression, as a distinct biological

subtype within HER2-negative breast cancer, affect the rate of pathological complete response to neoadjuvant chemotherapy and long-term survival outcomes in early-stage patients, compared to HER2-zero tumors?

**Knowledge Generated**: In a retrospective cohort study involving 934 patients, those with HER2-low tumors showed lower rates of pathological complete response compared to HER2-zero tumors, especially among hormone receptor–positive cases. Nonetheless, five-year disease-free and overall survival rates were comparable between the HER2-low and HER2-zero groups.

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