scientific reports

Check for updates

OPEN

Clinical characteristics and prognostic impact of HER2 low expression in breast cancer subtypes from a Brazilian real-world cohort

André Mattar[1,2✉], Marcelo Antonini[2,3], Francisco Pimentel Cavalcante[2,4], Felipe Zerwes[2,5], Eduardo Camargo Millen[2,6], Fabricio Palermo Brenelli[2,7], Antônio Luiz Frasson[2,8], Marcelo Madeira[9], Marina Diógenes Teixeira[1,3], Andressa Gonçalves Amorim[3], Larissa Chrispim de Oliveira[1], Leonardo Ribeiro Sorares[10], Gil Facina[11], Rogerio Fenile[12], Ruffo Freitas-Júnior[10,13], Renata Arakelian[1,14], Marcela Bonalumi dos Santos[1,15], Henrique Lima Couto[16], Marcellus do Nascimento Moreira Ramos[3], Leandro Ladislau Alves[17], Júlio Antonio Pereira de Araújo[1,18], Renata Montarroyos Leite[15,19] & Luiz Henrique Gebrim[20]

Breast cancer (BC) is the most prevalent cancer among Brazilian women, yet related data remain limited. The HER2-low classification has gained significance with the advent of targeted therapies. This study aimed to assess survival outcomes of HER2-low BC compared to other subtypes in a real-world Brazilian cohort. We analyzed data from 8,485 breast cancer patients treated at Pérola Byington Hospital between 2010 and 2019. Overall survival (OS) was the primary endpoint, stratified by cancer subtype. The t-test and chi-square test evaluated variable associations, while multivariate analysis calculated odds ratios and 95% confidence intervals. Cox regression assessed survival, and Kaplan–Meier curves illustrated OS differences. The patients with HER2-low breast cancers showed significantly better overall survival than those with the triple-negative subtype ($p < 0.01$). However, they had significantly poorer overall survival than those with the Luminal A-like subtype ($p < 0.01$). The patients with triple-negative HER2-low disease had a higher risk of mortality than those with HER2-0 ($p < 0.01$). Finally, patients who achieved pathological complete response experienced significantly better overall survival than those who did not ($p < 0.01$). Our findings highlight triple negative HER2-low BC as a distinct subtype identifiable via standard immunohistochemistry, beyond just biomarker status. The study underscores the prognostic diversity among BC subtypes and emphasizes the importance of personalized treatment strategies.

Keywords HER2-low breast cancer, Survival outcomes, Real-world data, Prognostic implications

[1]Centro de Referência da Saúde da Mulher – Hospital da Mulher, Avenida Rio Branco, 1080, São Paulo, SP 01206-001, Brazil. [2]BBREAST Group: Brazilian Breast Cancer Associate Research Team, São Paulo, Brazil. [3]Hospital do Servidor Público Estadual Francisco Morato de Oliveira, São Paulo, SP, Brazil. [4]Hospital Geral de Fortaleza, Fortaleza, CE, Brazil. [5]Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil. [6]Américas Oncologia, Rio de Janeiro, RJ, Brazil. [7]Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil. [8]Hospital Albert Einstein, São Paulo, SP, Brazil. [9]Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo, SP, Brazil. [10]Universidade Federal de Goias, Goiania, GO, Brazil. [11]Universidade Federal de São Paulo, São Paulo, SP, Brazil. [12]Hospital Ipiranga, São Paulo, SP, Brazil. [13]CORA Advanced Center for Breast Cancer Diagnosis, Federal University of Goias, São Paulo, Brazil. [14]DASA Oncologia, São Paulo, Brazil. [15]Oncoclínicas, São Paulo, Brazil. [16]Redimama - Redimasto, Belo Horizonte, MG, Brazil. [17]Head of Evidence Generation, Bristol Myers Squibb,

São Paulo, Brazil. [18]OncoCenter, São Paulo, SP, Brazil. [19]Universidade Federal de Sergipe, Aracaju, SE, Brazil. [20]Hospital Beneficência Portuguesa, São Paulo, São Paulo, Brazil. ✉email: mattar.andre@gmail.com

Breast cancer (BC) is the most prevalent malignant tumor in women and remains the leading cause of cancer-related mortality worldwide[1], including in Brazil[2] with significant variability in biological characteristics and clinical outcomes[3]. This heterogeneity reflects the presence of distinct tumor subtypes, each with unique prognostic and therapeutic implications[4,5]. In 2022, the World Health Organization reported that approximately 2.3 million women were diagnosed with BC, leading to approximately 670,000 deaths globally[6].

The heterogeneity of BC is reflected in its various subtypes, each with unique prognostic and therapeutic implications[7–10]. In clinical practice, immunohistochemistry is commonly used to classify BC subtypes based on hormone receptor status, human epidermal growth factor receptor 2 (HER2) expression, and proliferation markers such as Ki67 [11–13]. This classification plays a critical role in guiding treatment decisions and optimizing patient management.

BC is classified as HER2-positive when HER2 expression is scored as 3 + on immunohistochemistry (IHC) or 2 + with confirmed gene amplification via in situ hybridization (ISH), typically associated with more aggressive tumor behavior. For these tumors, anti-HER2 targeted therapies are strongly recommended[14,15]. Conversely, tumors with IHC scores of 0 or 1+, or 2 + without ERBB2 amplification by ISH, have been historically categorized as HER2-negative due to their lack of response to conventional anti-HER2 therapies[10,16–18].

Recent evidence suggests the need for reclassification within HER2-negative tumors [14]. The term HER2-low has been proposed to describe tumors with IHC scores of 1 + or 2 + without gene amplification, accounting for approximately 50–55% of all primary BCs. [15,18–21]. While traditional HER2-targeted therapies have shown limited benefit in HER2-low tumors, emerging treatments have demonstrated promising results in this subset, highlighting the potential for novel therapeutic strategies[18,22,23].

Despite growing interest in HER2-low BC, there remain significant gaps in understanding its epidemiology, treatment responses, and long-term outcomes. With the advent of new targeted therapies, a comprehensive characterization of HER2-low BC is critical to refining treatment strategies and improving patient outcomes.

Real-world data (RWD) provide valuable healthcare insights derived from diverse sources outside traditional clinical trials[24] and can complement findings from randomized controlled studies[25].

In Brazil, BC is the most commonly diagnosed cancer among women[2], yet data on its subtypes remain limited. According to a 2021 Interfarma report[26], Brazil accounts for only 2.4% of global clinical trials, underscoring the need for locally generated evidence.

Methods

Study design

This retrospective cohort study leveraged RWD from the Women's Hospital in São Paulo, Brazil (formerly known as Pérola Byington Hospital), a leading reference center for breast and gynecological cancer in the country. The analysis was conducted using secondary data from the institutional database and was approved by the ethics committee. The study adhered to ISPE/ISPOR guidelines for exploratory real-world research[27]. Data from women diagnosed and treated at the hospital between January 2010 and December 2019 were included in the analysis.

Patients selection

Women aged 18 years or older with a confirmed diagnosis of breast cancer (BC) stage I-IV during the study period were included. All patients underwent immunohistochemical testing to determine the tumor subtype at a local practice. First, we did not include multicentric or multifocal tumors and any recurrence diagnosed in the breast. Exclusion criteria encompassed patients diagnosed with benign tumors, carcinoma in situ, bilateral BC, male patients with cancer, individuals enrolled in clinical trials, loss of follow up and those with inconclusive or missing immunohistochemical data.

Patients with incomplete immunohistochemical or clinical follow-up data were excluded from the analysis. An exploratory evaluation indicated that data were missing at random (MAR). To assess potential selection bias, we compared baseline characteristics between included and excluded patients (see Table S1).

Subtype characterization

Molecular subtype classification was based on the expression of estrogen receptor (ER), progesterone receptor (PR), and HER2, assessed through routine immunohistochemistry (IHC). The cutoff for ER and PR positivity was set at ≥ 1% of tumor cells displaying nuclear staining[11]. Hormone receptor status was determined in two ways: classified as positive or negative (≥ 1% ER or PR positivity) or using the Allred score (≥ 3) [28] when percentage data were unavailable. The Ki-67 proliferation index was used to distinguish luminal A-like from luminal B-like subtypes, applying a cutoff of 14% [28]. ER, PR, Ki-67 and HER2 status were obtained from historical pathology reports at the time of diagnosis; no centralized or repeated testing was performed.

Breast cancer subtypes were defined as follows:

HER2-positive (HER2+): Tumors with HER2 overexpression, identified as IHC 3 + or IHC 2 + with gene amplification confirmed by in situ hybridization (FISH or CISH). The HER2 + cohort was also stratified according to hormone receptor status (HR) into HER2-positive/HR + and HER2-positive/HR– subgroups.

Triple-negative (TNBC): Tumors lacking ER, PR, and HER2 expression.

Luminal A-like: HER2-negative tumors with ER and PR positivity and a low Ki-67 index (< 14%) [21].

Luminal B-like: HER2-negative tumors with ER and/or PR positivity and a high Ki-67 index (≥ 14%) [21].

The luminal subtypes were grouped and stratified according to HER status into HR+/ HER2-0 and HR+/ HER2-low.

HER2-low: Tumors exhibiting HER2 expression of IHC 1 + or IHC 2 + without gene amplification confirmed by in situ hybridization[18]. All analyses were conducted based on the reclassification. TNBC was also stratified according to HER status into TNBC HER2-0 and TNBC HER2-low.

Treatment protocol

Patients received standard treatment according to their immunohistochemical profile. Luminal A-like and B-like patients with high risk or that undergo neoadjuvant chemotherapy were treated with regimens including doxorubicin, cyclophosphamide, and taxanes. HER2-positive patients received the same regimen with the addition of trastuzumab, while TNBC patients were treated with doxorubicin, cyclophosphamide, taxanes and more recently platinum-based chemotherapy. As a public institution CDK 4/6 inhibitors and immunotherapy for TNBC remain unavailable. Trastuzumab was introduced for advanced disease in 2011 and later incorporated into the neoadjuvant and adjuvant settings in 2013. In 2018, the combination of trastuzumab and pertuzumab became available exclusively for metastatic disease, while T-DM1 has not yet been introduced in any clinical.

Ethics

The Institutional Review Board of Perola Byington Hospital approved the use of patient data before the study was commenced under the reference number CAAE 7238317.6.0000.0069. This was a retrospective study and the requirement for informed consent was waived because of that. This study was conducted according to the principles of the Declaration of Helsinki, including the protection of patient confidentiality.

Outcomes

The primary outcome was overall survival (OS), which was defined as the duration (in months) from the date of diagnosis to the date of death (from any cause). The patients were considered alive at the date of their last consultation (diagnosis date and last consultation date).

The secondary outcomes were overall survival of patients with HER2-low BC with pCR relative to those without pCR and the impact of Ki67 levels of > 14 on survival of the patients with HER2-low BC and survival of the patients with HER2-low BC, with those with positive and negative hormone receptor statuses differentiated.

Statistical analysis

Descriptive data analysis was performed using absolute and relative frequencies and dispersion measures. The endpoint was overall survival stratified according to the cancer subtype. Both t-test and chi-squared test were used to compare the subtypes of BC. Cox regression was used for survival analysis using the log-rank method, and the results were presented in a survival graph using the Kaplan–Meier method. R software version 4.1.1 was used to perform all analyses; p-values of <0.05 denoted statistical significance. Multivariate analysis was conducted on key variables using a Cox proportional hazards model. We further conducted a stratified survival analysis within the HER2-low subgroup according to hormone receptor status (positive vs. negative), using Kaplan–Meier estimates and the log-rank test to compare overall survival.

Additionally, an exploratory analysis was performed to evaluate the distribution of tumor subtypes and receipt of treatment modalities across self-reported ethnic groups, in order to assess potential disparities related to ancestry and healthcare access.

Results

Overall, this study included 12,283 BC diagnoses in 11,069 patients. After excluding patients with missing values for baseline characteristics of interest, 8,796 BC diagnoses (Fig. 1). A comparative analysis showed that excluded patients were generally older and more likely to have unknown stage or treatment status. Full characteristics are detailed in Table S1, and these differences were taken into account when interpreting our findings.

A flowchart of the patients after a reclassification in HER2-low is shown in Fig. S1 from a traditional classification into a refined categorization incorporating HER2-low status. Initially, the cohort of 8,796 patients was categorized into four conventional subtypes: HER2-positive (17.49%), triple-negative (21.13%), Luminal A-like (19.95%), and Luminal B-like (41.43%). With the revised classification, the proportion of HER2-positive cases remained relatively unchanged at 17.46%, while the percentage of triple-negative cases decreased to 18.07%, likely due to the reclassification of some cases as HER2-low. Similarly, the proportion of Luminal A-like and Luminal B-like cases declined to 15.11% and 28.62%, respectively, as a significant portion of these tumors were reclassified as HER2-low. The newly introduced HER2-low category accounted for 15.29% of cases in hormone receptor-positive tumors and 5.46% in hormone receptor-negative tumors.

To better identify and evaluate the behavior of each subtype, we performed separate analyses comparing HR-positive/HER2-low vs. HR-positive/HER2-0 and TNBC/HER2-low vs. TNBC/HER2-0 groups.

To explore the potential impact of evolving HER2 testing standards, we stratified HER2-low prevalence across three diagnostic periods: 2010–2013, 2014–2016, and 2017–2019. HER2-low classification increased progressively from 17.8% in 2010–2013 to 21.6% in 2014–2016, and 22.6% in 2017–2019, suggesting a potential influence of improved testing practices and growing recognition of this subgroup.

Patients with incomplete immunohistochemical or clinical follow-up data were excluded from the analysis. To assess whether the exclusion introduced systematic bias, we compared baseline characteristics between included and excluded patients using independent t-tests for age and chi-square tests for categorical variables (clinical stage, molecular subtype, and hormone receptor status) suggesting that data was missing at random (See Table S1).

The characteristics of the patients with breast cancer at diagnosis are shown in Table 1. Most of the included patients had clinical stage II disease (approximately 40%). Regarding the self-reported ethnicity distribution,

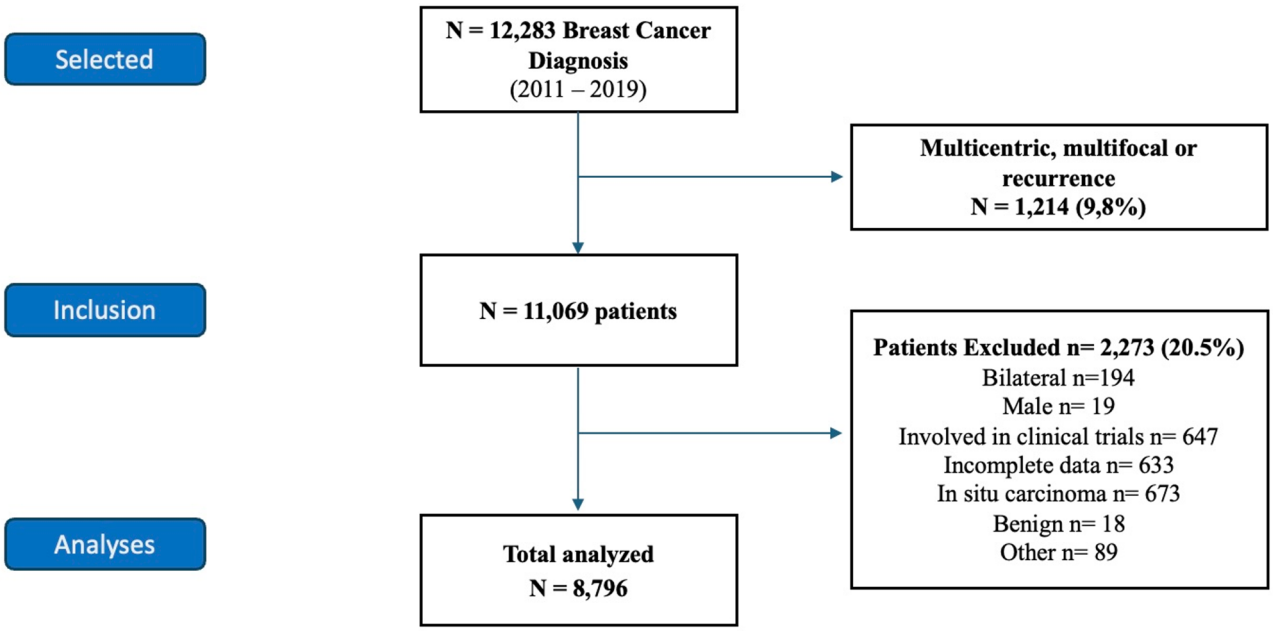

Fig. 1. Patients enrolled in the study.

most participants declared themselves as white (54.4%). Blacks, Asians, and other ethnicities were less represented across all groups.

An exploratory analysis of ethnicity revealed statistically significant differences in the distribution of breast cancer subtypes ($p < 0.001$). Triple-negative tumors were more frequently observed among Black (21.5%) and Brown (24.6%) women compared to White women (16.4%). In contrast, Luminal A-like and Luminal B-like subtypes were more prevalent among White women. However, there were no statistically significant differences in current alcohol consumption between groups ($p = 0.791$), and treatment variables such as recurrence rates and menopausal status varied by subtype but not consistently by ethnicity. While these findings suggest a potential interplay between ethnicity and tumor biology, further investigation using granular socioeconomic indicators would be needed to fully assess disparities in care access and outcomes.

The Table 2 presents the distribution of breast cancer subtypes according to clinical stage. The Luminal A-like subtype is predominantly diagnosed at early stages, with 36.87% in stage I and 38.98% in stage II, while later stages are less frequent (14.52% in stage III and 1.43% in stage IV). In contrast, Luminal B-like has a broader distribution, with most cases in stage II (40.68%), followed by stage III (27.81%) and stage I (22.84%), suggesting a more aggressive profile. The triple-negative subtype exhibits a distinct pattern, with the highest proportion in stage III (37.89%) and stage II (36.25%), while stage I cases are lower (16.68%). Stage IV cases remain relatively uncommon across all subtypes, ranging from 1.43% in Luminal A-like to 4.36% in HER2+. A small proportion of cases in each subtype have missing stage data, varying from 1.24% in Luminal A-like to 6.23% in triple-negative.

The patients with the Luminal A-like subtype had more favorable OS (mean OS = 106 months) than those with the HER2 + subtype. Those with the triple-negative subtype had the least favorable OS (mean OS = 96 months). The patients with HER2-low BC had better survival outcomes than those with the triple-negative subtype (HR: 0.38, 95% CI: 0.23–0.53, $p < 0.01$). In contrast, the patients with the Luminal A-like subtype had significantly better OS than those with the HER2-low subtype with a hazard ratio (HR) of -0.58 (95% CI: -0.77 to -0.38, $p < 0.01$) (Fig. 2) reveals the OS differences between the HER2-low subtype and the other BC subtypes.

Among patients with HER2-low breast cancer, survival was further stratified by hormone receptor status. HR-positive patients had significantly better overall survival compared to their HR-negative counterparts. At 60 months, the estimated survival probability was approximately 72% for HR-positive and 87% for HR-negative HER2-low patients, indicating substantial prognostic heterogeneity within this subgroup. The median survival for patients with HRs was 101 months, whereas that for those without HRs was 90 months. (HR: 0.39, 95% CI: 0.32 to 0.48; $p < 0.01$) – Fig. 3.

No statistically significant differences in survival were observed among the patients with the other subtypes.

To better assess the influence of HER2-low status on 5-year survival outcomes, stage IV cases were excluded from the analysis. We compared HR+/HER2-low vs. HR+/HER2-0, showing no significant difference (79.0% vs 78.4%; p = 0.64; 95% CI − 1.9 to + 3.1%). Conversely, among TNBC patients, HER2-low tumors demonstrated significantly worse survival compared with TNBC/HER2-0 (55.4% vs. 67.7%; p < 0.001; 95% CI − 18.9 to − 5.8%) as shown on Fig. 4.

In the univariate analyses patients with HER2-low BC who achieved pCR experienced better overall survival than those who did not achieve pCR [HR: 0.36 (95% CI: 0.27 to 0.47); $p < 0.01$]. The median survival durations were 104 and 85 months for those with and without pCR, respectively (See Fig. S2).

	Overall population *n* = 8,796 (100%)	HER2+ *n* = 1,536 (17.46%)	Triple-negative *N* = 1,589 (18.07%)	Luminal A-like *N* = 1,329 (15.11%)	Luminal B-like *N* = 2,517 (28.62%)	HER-low *n* = 1,825 (20.75%)	*p*-value*
Age (years) mean [SD]	56.10 [13.41]	52.83 [12.63]	54.73 [13.77]	59.87 [13.07]	56.59 [13.35]	56.60 [13.28]	0.917
Ethnic origin							
White	4,786 (54.21)	814 (17.01)	785 (16.40)	761 (15.90)	1,387 (28.98)	1,039 (21.71)	< 0.001
Black	735 (8.36)	133 (11.29)	158 (21.50)	83 (11.29)	217 (29.52)	144 (18.10)	< 0.001
Asian	105 (1.19)	17 (16.19)	11 (10.48)	21 (20.00)	31 (29.52)	25 (23.81)	< 0.001
Brown	2,841 (32.30)	505 (17.78)	554 (24.62)	426 (14.99)	810 (28.51)	546 (19.22)	< 0.001
Other	329 (3.74)	67 (20.36)	81 (24.62)	38 (11.55)	72 (21.88)	71 (21.58)	0.001
Current smoker	961 (10.93)	166 (17.27)	157 (16.34)	140 (14.56)	270 (28.10)	228 (23.72)	< 0.001
Current drinker	63 (0.72)	12 (19.05)	9 (14.29)	13 (20.63)	14 (22.22)	15 (23.81)	0.791
Recurrence	1,225 (13.93)	197 (16.08)	241 (19.67)	189 (15.43)	329 (26.86)	269 (21.96)	< 0.001
Menopausal status							
Pre	2,982 (33.91)	633 (21.22)	594 (19.01)	238 (7.98)	847 (28.40)	580 (19.45)	< 0.001
Post	5814 (66.09)	903 (15.53)	995 (17.11)	1,001 (17.22)	1,670 (28.72)	1,245 (21.41)	< 0.001

Table 1. Characteristics of patients with breast cancer at diagnosis. HER2-low (HER2 1 + or 2 + with ISH negative); HER2+ (HER2 3 + or ISH positive), Luminal A-like (hormonal receptor positive, at least 1% and Ki67 below 14%); Luminal B-like (hormonal receptor positive, at least 1% and Ki67 equal or more 14%). * chi-square.

	Overall population *n* = 8,796 (100%)		HER2+ *n* = 1,536 (17.46%)		Triple-negative *N* = 1,589 (18.07%)		Luminal A *N* = 1,329 (15.11%)		Luminal B *N* = 2,517 (28.62%)		HER-low *n* = 1,825 (20.75%)		
Stage	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	***p*-value***
I	2078	23.6	277	3.15	265	3.01	490	5.57	575	6.54	470	5.34	0.917
II	3437	39.3	582	6.62	576	6.55	518	5.89	1024	11.64	737	8.38	< 0.001
III	2542	28.9	538	6.12	602	6.84	193	2.19	700	7.96	509	5.79	< 0.001
IV	279	3.2	67	0.76	47	0.53	19	0.22	95	1.08	51	0.58	< 0.001
Missing stage	461	5.0	72	0.82	99	1.03	109	8.2	123	1.39	58	0.66	< 0.001

Table 2. Distribution of breast cancer subtypes according to clinical stage. HER2-low (HER2 1 + or 2 + with ISH negative); HER2+ (HER2 3 + or ISH positive), Luminal A-like (hormonal receptor positive, at least 1% and Ki67 below 14%); Luminal B-like (hormonal receptor positive, at least 1% and Ki67 equal or more 14%). * chi-square.

For the HER2-low subtype, low Ki67 levels (< 14%) were associated with improved OS (HR, 0.63; 95% CI: 0.37–0.89) – Fig. 5.

A multivariate analysis (Fig. 6) was conducted and patients HER2-low that presented stage I and II patients had significantly better survival compared to Stage III. Stage I: HR = 0.11 (95% CI: 0.07–0.18, $p < 0.001$). Stage II: HR = 0.20 (95% CI: 0.13–0.31, $p < 0.001$).

Both adjuvant and neoadjuvant chemotherapy were associated with a significant reduction in mortality risk. Adjuvant chemotherapy: HR = 0.32 (95% CI: 0.18–0.56, $p < 0.001$). Neoadjuvant chemotherapy: HR = 0.43 (95% CI: 0.30–0.62, $p < 0.001$).

Lack of hormone therapy correlates with worse outcomes HR = 0.33 (95% CI: 0.26–0.41, $p < 0.001$). Patients who did not receive radiotherapy also demonstrated worse survival HR = 0.56 (95% CI: 0.45–0.70, $p < 0.001$). Patients who achieved pathological complete response (pCR) after neoadjuvant therapy showed a trend toward improved survival, though not statistically significant HR = 0.83 (95% CI: 0.48–1.41, $p = 0.483$). Non-Black patients did not exhibit a significant difference in survival HR = 0.93 (95% CI: 0.75–1.15, $p = 0.492$).

Discussion

Historically, HER2-low breast cancer was grouped within the HER2-negative category and did not influence treatment decisions. However, recent evidence has highlighted its potential biological and therapeutic relevance[21,29]. However, this paradigm shifted significantly after the DESTINY-Breast04 trial. This study randomized 557 patients with HER2-low metastatic BC in a 2:1 ratio to receive either trastuzumab deruxtecan (T-DXd) or the physician's choice of chemotherapy. All patients had previously received 1–2 lines of chemotherapy

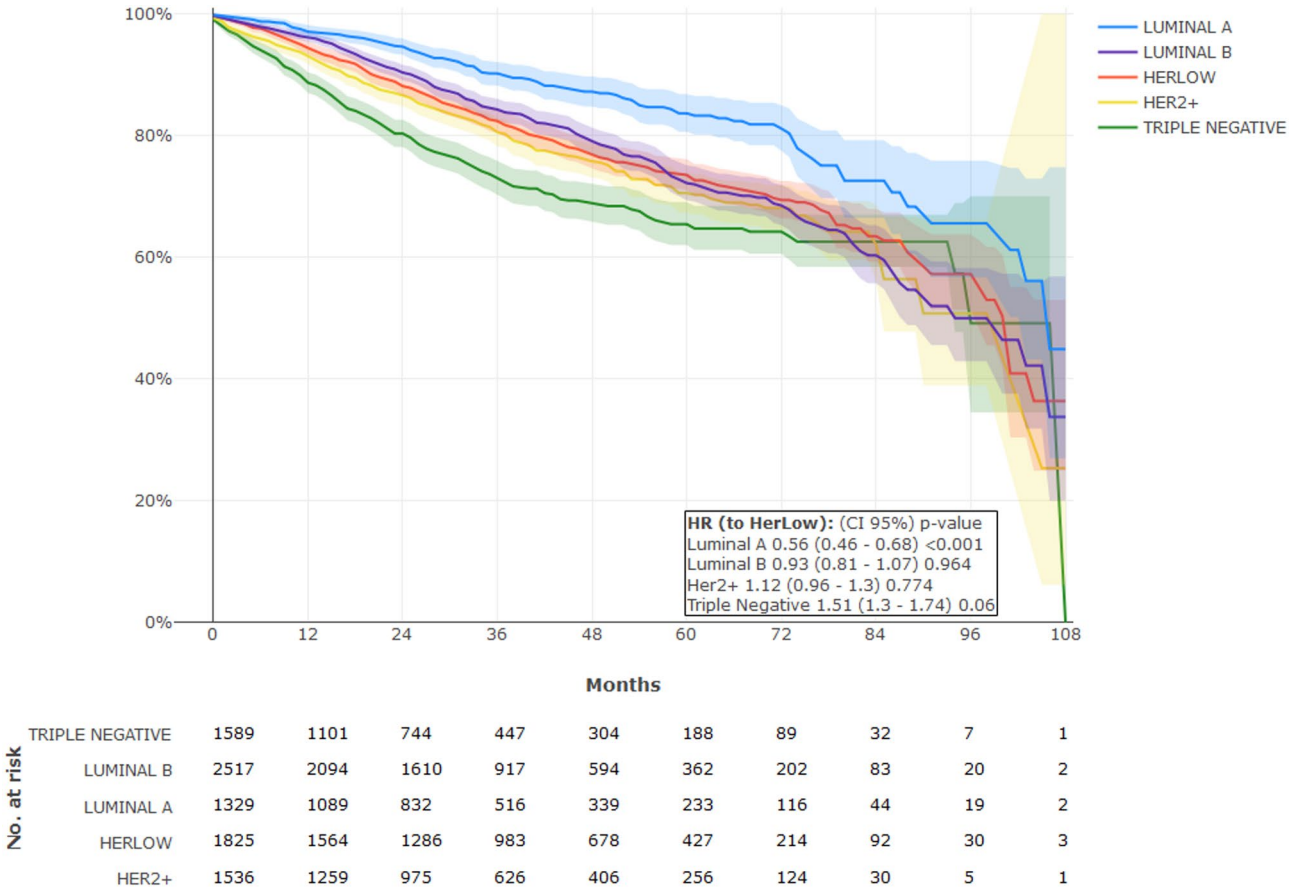

Fig. 2. OS differences between the HER2-low subtype and the other BC subtypes.

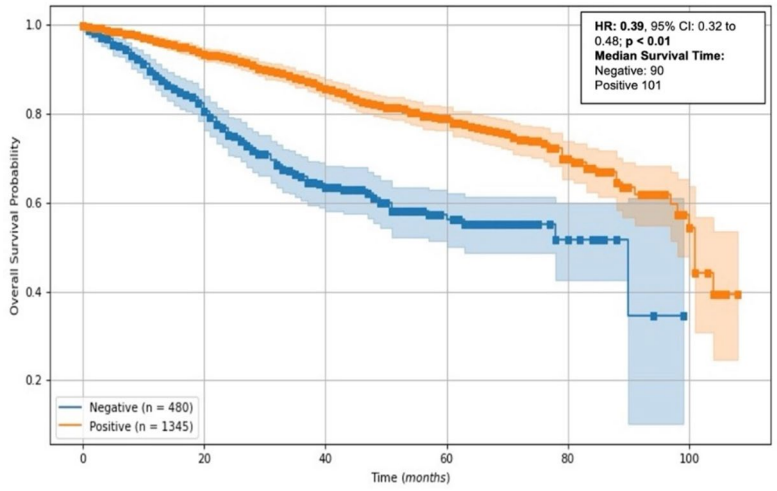

Fig. 3. OS in HER2-low BC (positive and negative HR status).

in the metastatic setting, and endocrine resistance was considered in HR-positive cases. The trial demonstrated the superiority of T-DXd over standard chemotherapy, reinforcing HER2-low as a clinically relevant category[17].

Our findings suggest distinct survival patterns among breast cancer subtypes, supporting the notion that HER2-low breast cancer may represent a clinically and biologically unique disease entity. Patients with TNBC/HER2-low breast cancer had significantly better survival than those with TNBC/HER2-0. However, in patients with HR-positive this difference was not observed. No statistically significant survival differences were observed

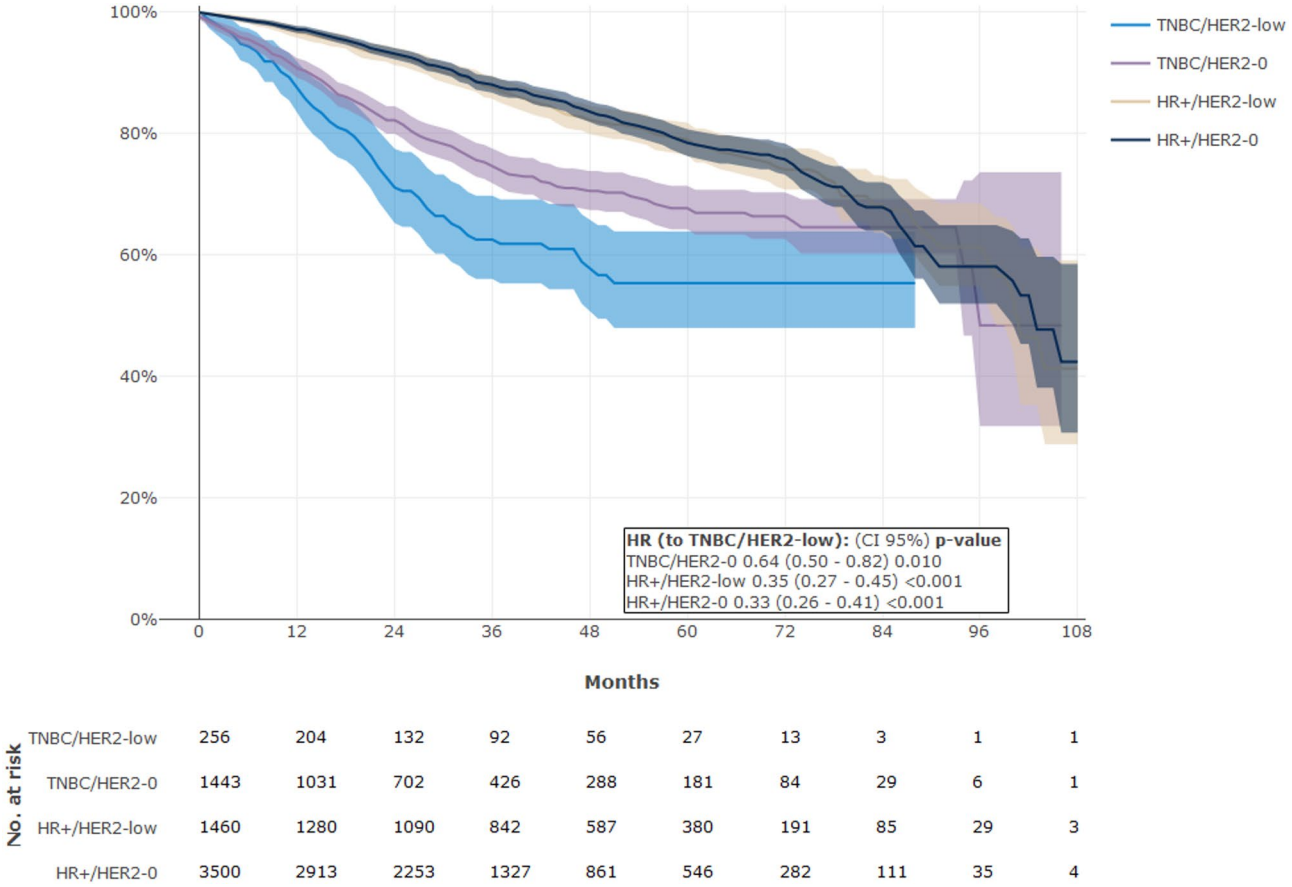

Fig. 4. OS according to HER2-low status in TNBC and HR-positive subtypes.

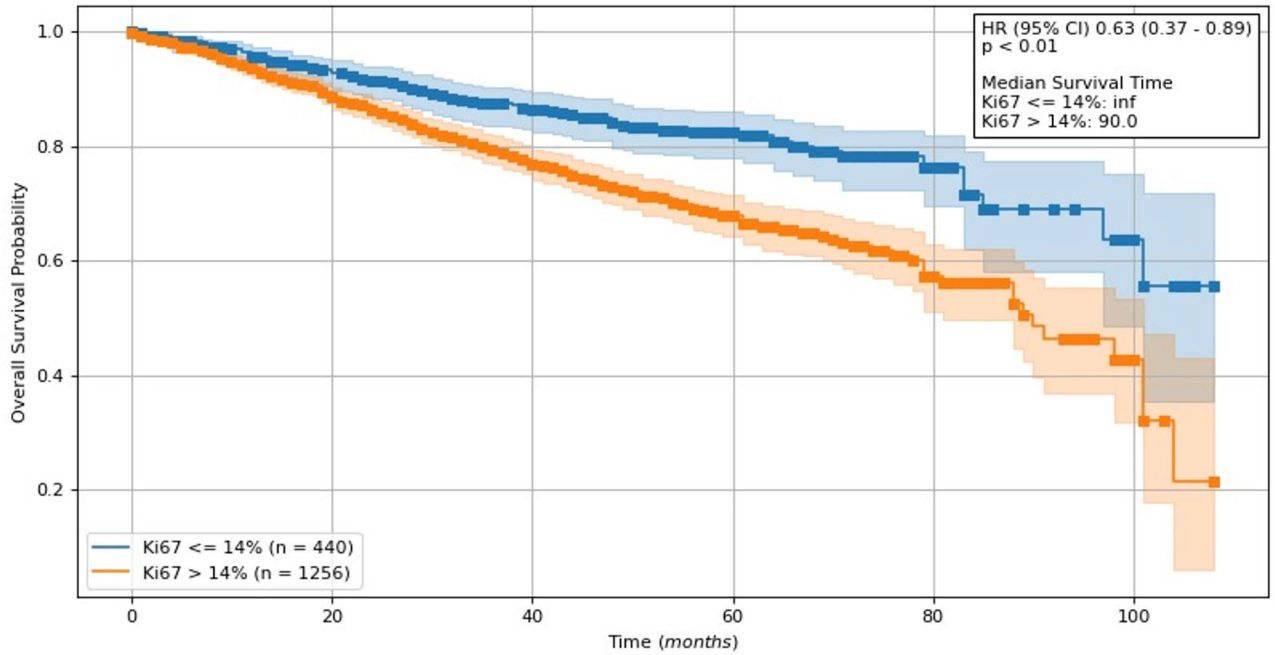

Fig. 5. Effect of Ki67 levels on OS in HER2-low subtype.

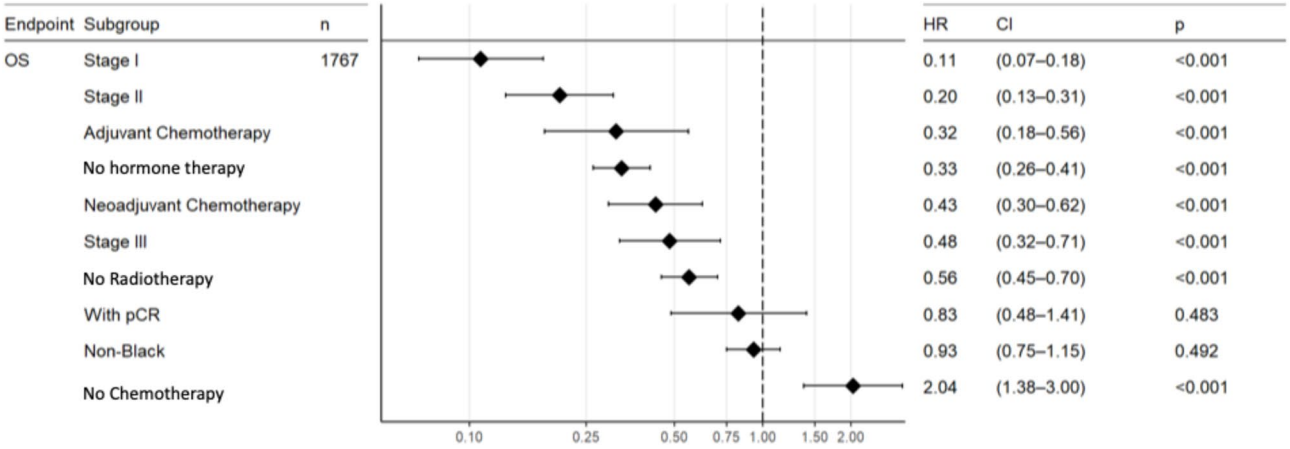

Fig. 6. OS multivariate analyses in HER2-low.

among the other subtypes. Additionally, our study indicated that hormone receptor negative patients had a higher risk of mortality than HR-positive patients, reinforcing the prognostic relevance of HR status.

While some studies have argued that HER2-low is not a distinct biological entity—suggesting its features are largely driven by HR status—our data challenge this assumption[14,30]. In our large and diverse Brazilian cohort, HER2-low tumors demonstrated clinical characteristics and survival trajectories that remained distinct even after stratification by HR status, including within luminal A-like and luminal B-like subgroups. These findings suggest that HER2-low tumors may represent a unique clinical phenotype within the broader breast cancer landscape, warranting further investigation into their biological underpinnings and therapeutic implications. These findings are consistent with recent Brazilian data by Antonini et al. (2024), who also demonstrated the prognostic relevance of HER2-low status in a real-world cohort[31].

Although our study was not designed to assess treatment efficacy, the distinct survival patterns observed in TNBC/HER2-0 tumors suggest they may respond differently to adjuvant therapies. These patients had better outcomes than those with TNBC/HER2-0. This intermediate prognosis may indicate a window for personalized strategies for future studies.

Our cohort exhibited distinct characteristics compared to prior reports. Notably, only 20.75% of patients had HER2-low BC, which is considerably lower than the 40–50% prevalence reported in previous studies[19,32]. In addition, we observed a temporal increase in HER2-low classification from 2010 to 2019, consistent with global trends. This pattern likely reflects progressive improvements in immunohistochemical testing accuracy and the increasing recognition of HER2-low as a clinically meaningful category, especially following the updated ASCO/CAP guidelines in 2013 and the emergence of HER2-targeted ADCs.

Several factors could explain this discrepancy: (1) Variability in IHC methodologies – Differences in antibodies and testing protocols could influence HER2 classification; (2) Challenges in distinguishing HER2 0 vs. HER2 1+ – Until recently, differentiating HER2 0 from HER2 1 + had little clinical relevance, possibly leading to misclassification in pathologic assessments; (3) Pathology reassessment discrepancies – A study reported that only 15% of cases initially categorized as HER2 0 remained unchanged upon central review, with 76% being reclassified as HER2-low[32] highlighting the potential subjectivity in HER2 scoring; In our cohort, 3,109 tumors were initially classified as HER2 0. Applying the 76% reclassification rate observed in the literature suggests that approximately 2,363 of these cases might have been HER2-low, which would increase our estimated prevalence to nearly 47%, aligning with international data. (4) Tissue sample variability – Differences between biopsy and surgical specimens could influence HER2 status, as it has been demonstrated before[33] and finally (5) Study population differences – Our sample slightly differed from other Brazilian studies[3,32,34]. For example, in the AMAZONA retrospective cohort, the mean patient age was 54 years, 39.1% were premenopausal, and most had Stage II (53.5%) or Luminal A-like (49.4%) BC. In contrast, our cohort had a mean age of 56 years, with a majority having Stage II and Luminal B-like (28.62%) [3]. Although our prevalence was lower than in prior studies, our cohort provides valuable real-world insights specific to a Brazilian population.

Consistent with previous studies, HER2-low BC had better OS than TNBC[14,35,36]. However, Luminal A-like patients had significantly better OS than HER2-low patients, suggesting a prognostic hierarchy. The pCR rates varied by subtype, and our findings align with a recent real-world study demonstrating the importance of trastuzumab in HER2 + BC for achieving pCR and its impact on OS. Several studies have investigated the association between pCR and OS, highlighting its prognostic significance, particularly in TNBC and HER2 + BC [37,38].

A systematic review of 52 studies involving 27,895 patients showed that achieving pCR significantly improved event-free survival and OS, with outcomes being independent of adjuvant chemotherapy. These findings emphasize the role of tumor biology and micrometastatic disease clearance in survival[39]. Our data align with this evidence, reinforcing that HER2-low BC patients who achieve pCR have significantly better OS. A recent Brazilian real-world study corroborated these findings[40].

However, some studies report conflicting results. For example, a 2021 study found no significant differences in response rates or recurrence risk between HER2-low and HER2-negative BC in patients receiving neoadjuvant chemotherapy[32]. This highlights the need for further research to clarify the clinical relevance of HER2-low status.

Our findings support the hypothesis that HER2-low BC may be a distinct biological subtype, consistent with the work of Denkert et al., who reported unique clinicopathological features, reduced chemotherapy responsiveness, and improved survival compared to HER2 0 tumors[41]. This is further supported by Schettini et al., who recently demonstrated biological heterogeneity within HER2-low tumors, particularly among young women with germline BRCA1/2 mutations, suggesting that germline predisposition may further influence HER2 expression profiles and therapeutic responsiveness[42]. However, other studies challenge this notion. Tarantino et al. (2022)[30], in a cohort of 5,235 HER2-negative BC patients, observed that most clinicopathologic differences between HER2 0 and HER2-low could be attributed to HR status. After adjusting for HR status, HER2-low and HER2 0 had no significant prognostic differences, casting doubt on whether HER2-low should be considered a separate entity.

Our findings also underscore the prognostic heterogeneity within the HER2-low subgroup. When stratified by hormone receptor status, HER2-low/HR-positive patients demonstrated markedly better overall survival than HER2-low/HR-negative patients. This aligns with emerging literature suggesting that HER2-low tumors do not represent a uniform biological entity, and that hormone receptor co-expression remains a dominant driver of outcome. These results reinforce the need to interpret HER2-low status in conjunction with HR status, especially when considering treatment selection and trial eligibility in this evolving landscape.

Our multivariate analysis confirms that early-stage diagnosis, chemotherapy (both neoadjuvant and adjuvant), hormone therapy, and radiotherapy significantly improve OS. Patients who did not receive these treatments had notably higher mortality risks, emphasizing the need for comprehensive, individualized treatment strategies.

Although our multivariate analysis accounted for several prognostic variables—including tumor stage, hormone receptor status, and receipt of systemic therapies—some potentially important confounders could not be evaluated due to limitations of the dataset. Specifically, information on comorbidities, performance status, and treatment adherence (such as compliance with endocrine therapy) was not systematically recorded. Additionally, novel agents such as CDK4/6 inhibitors, which are not routinely available in the Brazilian public healthcare system, were not included in our treatment variables. These unmeasured factors may influence survival outcomes and should be considered in future prospective or registry-based studies with more granular clinical data.

This study has several strengths, including the use of a large real-world cohort representing diverse BC cases across Brazil, along with an extended follow-up period that allowed for a robust overall survival analysis. However, some limitations should be considered. The retrospective design inherently introduces biases, and variability in pathology assessments must be acknowledged, as ER, PR, HER2, and Ki-67 were determined from local pathology reports without central confirmation. Additionally, Ki-67 scoring was performed manually, without automated quantification, which may have introduced interobserver differences[43]. The long inclusion period during which treatment guidelines evolved could also be a limitation. However, given that the public healthcare system follows strict protocols, treatment regimens remained relatively stable. Notably, CDK 4/6 inhibitors and immunotherapy for TNBC remain unavailable, and chemotherapy (anthracyclines and taxanes) was the primary systemic therapy.

We recognize that excluding patients with missing data may introduce selection bias. Although the majority of baseline characteristics were comparable between included and excluded patients, we observed statistically significant differences in age and clinical stage, particularly stage II. These differences were modest and unlikely to meaningfully impact the overall findings, but they highlight the inherent limitations of retrospective real-world datasets. Importantly, the large sample size, diverse population, and robust statistical analyses support the generalizability of our results despite these minor imbalances.

Furthermore, changes in HER2 testing guidelines over time may have influenced classification. HER2-targeted treatment availability evolved over the years. Trastuzumab was introduced for advanced disease in 2011 and later incorporated into the neoadjuvant and adjuvant settings in 2013. This has impacted survival among Brazilian patients[44]. In 2018, the combination of trastuzumab and pertuzumab became available exclusively for advanced disease, while T-DM1 has not yet been introduced in any clinical scenario within the public healthcare system.

In addition to these limitations, we conducted an exploratory analysis to investigate potential disparities related to ethnicity and tumor subtype distribution. Our analysis revealed statistically significant differences across self-reported ethnic groups, with triple-negative tumors more frequently observed among Black and Brown women, and Luminal A-like and B-like subtypes more prevalent among White patients. These findings are consistent with previous literature suggesting that tumor biology may vary across ancestral backgrounds. Although we did not find significant differences in treatment receipt (e.g., chemotherapy, radiotherapy) or alcohol consumption, the absence of detailed socioeconomic data may limit interpretation. These patterns raise the possibility that ethnicity-related biological features — possibly compounded by unmeasured structural inequities — could influence disease behavior and outcomes. Future studies should aim to incorporate granular sociodemographic and genomic data to further elucidate these associations in diverse populations. Given the potential racial and ethnic differences in cancer biology, caution is warranted when extrapolating these findings to broader populations outside of Brazil.

Conclusion

The study revealed that HER2 expression significantly influences the overall survival rates of breast cancer patients in TNBC. The findings gleaned from this research offer valuable guidance for tailoring management strategies to the distinct subtypes of breast cancer, thereby enhancing patient outcomes. Furthermore, these

insights will inform the advancement of novel therapies tailored specifically for HER2-low breast cancers. Further prospective studies are warranted to validate these findings and optimize HER2-low treatment strategies.

Data availability

The datasets analyzed during the current study are available fully available upon request. Please contact [mattar.andre@gmail.com](mailto: mattar.andre@gmail.com) .

Received: 16 March 2025; Accepted: 3 December 2025
Published online: 12 December 2025

References

1. Bray, F. et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Cancer J. Clin.* **74** (3), 229–263 (2024).
2. Santos, M. O. et al. Estimativa de Incidência de Câncer no Brasil, 2023–2025. *Rev. Brasil. Cancerol.* **69** (1), e–213700 (2023).
3. Simon, S. D. et al. Characteristics and prognosis of stage I-III breast cancer subtypes in brazil: the AMAZONA retrospective cohort study. *Breast* **44**, 113–119 (2019).
4. Perou, C. M. et al. Molecular portraits of human breast tumours. *Nature* **406** (6797), 747–752 (2000).
5. Sørlie, T. et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U S A.* **98** (19), 10869–10874 (2001).
6. WHO. (World Health Organization) Global health estimates-Breast Cancer [Internet] (2022).
7. Holm, J. et al. Concordance of Immunohistochemistry-Based and gene Expression-Based subtyping in breast cancer. *JNCI Cancer Spectr.* **5**, 1 (2021).
8. Howlader, N. et al. Differences in breast cancer survival by molecular subtypes in the united States. *Cancer Epidemiol. Biomarkers Prev.* **27** (6), 619–626 (2018).
9. Schneeweiss, A. et al. Long-term efficacy analysis of the randomised, phase II TRYPHAENA cardiac safety study: evaluating Pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer. *Eur. J. Cancer.* **89**, 27–35 (2018).
10. Venetis, K. et al. HER2 Low, Ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. *Front. Mol. Biosci.* **9**, 834651 (2022).
11. Allison, K. H. et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *J. Clin. Oncol.* **38** (12), 1346–1366 (2020).
12. Curigliano, G. et al. Highlights from the 13th St Gallen international breast cancer conference 2013. Access to innovation for patients with breast cancer: how to speed it up? *Ecancermedicalscience* **7**, 299 (2013).
13. Wolff, A. C. et al. Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical Oncology/College of American pathologists clinical practice guideline focused update. *J. Clin. Oncol.* **36** (20), 2105–2122 (2018).
14. Schettini, F. et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *Npj Breast Cancer.* **7** (1), 1 (2021).
15. Zhang, H. & Peng, Y. Current Biological, pathological and clinical landscape of HER2-Low breast cancer. *Cancers (Basel)* **15**, 1 (2022).
16. Blows, F. M. et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Med.* **7** (5), e1000279 (2010).
17. Modi, S. et al. Trastuzumab Deruxtecan in previously treated HER2-Low advanced breast cancer. *N Engl. J. Med.* **387** (1), 9–20 (2022).
18. Tarantino, P. et al. HER2-Low breast cancer: pathological and clinical landscape. *J. Clin. Oncol.* **38** (17), 1951–1962 (2020).
19. Arias, V. E. A. et al. Assessment of HER-2 status in invasive breast cancer in Brazil. *Rev. Assoc. Med. Bras. 2017* **63** (7), 566–574 (1992).
20. Sørlie, T. Molecular portraits of breast cancer: tumour subtypes as distinct disease entities. *Eur. J. Cancer.* **40** (18), 2667–2675 (2004).
21. Wolff, A. C. et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO-College of American pathologists guideline update. *J. Clin. Oncol.* **41** (22), 3867–3872 (2023).
22. Li, Y. et al. Comprehensive characterization of HER2-low breast cancers: implications in prognosis and treatment. *EBioMedicine* **91**, 104571 (2023).
23. Popović, M. et al. HER2 low breast cancer: a new subtype or a Trojan for cytotoxic drug delivery? *Int. J. Mol. Sci.* **24**, 9 (2023).
24. Sherman, R. E. et al. Real-World Evidence - what is it and what can it tell us? *N Engl. J. Med.* **375** (23), 2293–2297 (2016).
25. Soni, P. D. et al. Comparison of Population-Based observational studies with randomized trials in oncology. *J. Clin. Oncol.* **37** (14), 1209–1216 (2019).
26. Interfarma. A importância da pesquisa clínica para o Brasil (internet) (2022). https://www.interfarma.org.br/wp-content/uploads/2023/08/Pesquisa-clinica-2022_atualizado.pdf.
27. Motheral, B. et al. A checklist for retrospective database studies–report of the ISPOR task force on retrospective databases. *Value Health.* **6** (2), 90–97 (2003).
28. Hammond, M. E. et al. American society of clinical Oncology/College of American pathologists guideline recommendations for immunohistochemical testing of Estrogen and progesterone receptors in breast cancer (unabridged version). *Arch. Pathol. Lab. Med.* **134** (7), e48–72 (2010).
29. Peiffer, D. S. et al. Clinicopathologic characteristics and prognosis of ERBB2-Low breast cancer among patients in the National cancer database. *JAMA Oncol.* **9** (4), 500–510 (2023).
30. Tarantino, P. et al. Evolution of low HER2 expression between early and advanced-stage breast cancer. *Eur. J. Cancer.* **163**, 35–43 (2022).
31. Antonini, M. et al. Analyzing neoadjuvant chemotherapy effects in HER2-Low breast cancer: real world data. *Cureus* **16** (5), e59652 (2024).
32. de Moura Leite, L. et al. HER2-low status and response to neoadjuvant chemotherapy in HER2 negative early breast cancer. *Breast Cancer Res. Treat.* **190** (1), 155–163 (2021).
33. Baez-Navarro, X. et al. HER2-Low breast cancer: Incidence, clinicopathologic features, and survival outcomes from Real-World data of a large nationwide cohort. *Mod. Pathol.* **36** (4), 100087 (2023).
34. Marques, A. D. et al. Analysis of breast cancer survival in a Northeastern Brazilian state based on prognostic factors: a retrospective cohort study. *PLoS One.* **17** (2), e0263222 (2022).
35. Wei, T. et al. Clinicopathologic characteristics and prognostic significance of HER2-low expression in patients with early breast cancer: a systematic review and meta-analysis. *Front. Oncol.* **13**, 1100332 (2023).
36. Xu, H. et al. Clinicopathological characteristics and prognosis of HER2-Low Early-Stage breast cancer: a Single-Institution experience. *Front. Oncol.* **12**, 906011 (2022).

37. Di Cosimo, S. et al. Moving HER2-low breast cancer predictive and prognostic data from clinical trials into the real world. *Front. Mol. Biosci.* **9**, 996434 (2022).
38. van Mackelenbergh, M. T. et al. Pathologic complete response and individual patient prognosis after neoadjuvant chemotherapy plus Anti-Human epidermal growth factor receptor 2 therapy of human epidermal growth factor receptor 2-Positive early breast cancer. *J. Clin. Oncol.* **41** (16), 2998–3008 (2023).
39. Spring, L. M. et al. Pathologic complete response after neoadjuvant chemotherapy and impact on breast cancer recurrence and survival: a comprehensive Meta-analysis. *Clin. Cancer Res.* **26** (12), 2838–2848 (2020).
40. Antonini, M. et al. Real-world evidence of neoadjuvant chemotherapy for breast cancer treatment in a Brazilian multicenter cohort: correlation of pathological complete response with overall survival. *Breast* **72**, 103577 (2023).
41. Denkert, C. et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *Lancet Oncol.* **22** (8), 1151–1161 (2021).
42. Schettini, F. et al. Characterization of HER2-low breast cancer in young women with germline BRCA1/2 pathogenetic variants: results of a large international retrospective cohort study. *Cancer* **130** (16), 2746–2762 (2024).
43. Fulawka, L. & Halon, A. Ki-67 evaluation in breast cancer: the daily diagnostic practice. *Indian J. Pathol. Microbiol.* **60** (2), 177–184 (2017).
44. Antonini, M. et al. Disparities in access to anti-HER2 therapies in neoadjuvant chemotherapy: a prognostic analysis based on real-world data comparing brazil's public and private healthcare systems. *Breast* **80**, 104417 (2025).

Acknowledgements
The authors acknowledge all the patients included in the trial.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by André Mattar. The first draft of the manuscript was written by André Mattar. A authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
The analyses and the medical writing were sponsored by Daichii Sankio.

Competing interests
André Mattar: Consulting or Advisory Role: AstraZeneca, Roche, Daichii Sankio, Novartis, and Lilly; Speakers' Bureau: AstraZeneca, Roche, Daichii Sankio, Novartis, and Lilly; and Research Funding: AstraZeneca, Roche, Daichii Sankio, Novartis, and Lilly.Leandro Ladislau Alves: Bristol Myers Squibb Brazil employee.The other authors have no conflicts of interest to declare.

Ethics approval
The Institutional Review Board of Perola Byington Hospital approved the use of patient data before the study was commenced under the reference number CAAE 7238317.6.0000.0069. This was a retrospective study and the requirement for informed consent was waived because of that. This study was conducted according to the principles of the Declaration of Helsinki, including the protection of patient confidentiality.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-31515-y.

Correspondence and requests for materials should be addressed to A.M.