![USCAP logo]

**ARTICLE**

# HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles

Huina Zhang [1,3 ✉], Hani Katerji[1,3], Bradley M. Turner [1], William Audeh[2] and David G. Hicks[1 ✉]

Recently, clinical trials have demonstrated promising efficacy for novel HER2-targeted therapies in HER2-low breast cancers, raising the prospect of including a HER2-low category (immunohistochemical [IHC] score of 1+, or 2+ with non-amplified in-situ hybridization [ISH]) in the HER2 evaluation of breast cancers. In order to better understand this newly-proposed HER2 category, we investigated the incidence, HER2 staining patterns, clinicopathologic features, and genomic profile of HER2-low breast cancers. HER2-stained slides of 281 consecutive breast cancers were re-reviewed and the clinicopathologic information, MammaPrint, and BluePrint results of these cases were retrospectively analyzed. HER2-low breast cancers were identified in 31% of cases and were more common in estrogen receptor (ER)-positive than ER-negative breast cancers (33.6% vs 15%, $p = 0.017$). HER2-low cancers were generally clinical stages I–II (79%), ER-positive (93.1%), had homogenous HER2 staining (59.2%), HER2 IHC score of 1+ (87.4%), ductal phenotype (81.6%), histologic grades of 1 or 2 (94.2%) and luminal molecular subtypes (94.3%). Three HER2-low patients received neoadjuvant chemotherapy and none of them achieved pathologic complete response. When compared to HER2-negative (IHC of 0+) and HER2-positive (IHC of 3+ or IHC of 2+ with amplified ISH) cancers, HER2-low breast cancers had significantly lower Ki-67 ($p = 0.03$ and $p < 0.01$, respectively) and higher ER positivity ($p = 0.01$ and $p = 0.03$, respectively). HER2-low breast cancers were less likely to be basal molecular subtype when compared to HER2-negative cancers ($p < 0.01$) and were less likely to have a HER2 molecular subtype when compared to HER2-positive cancers ($p < 0.01$). When adjusted for ER status, there was no significant difference on all the examined variables between HER2-low and HER2-negative groups. Our study provides valuable baseline characteristics of HER2-low breast cancers deriving from consecutive, real-world cases with a consensus confirmation of HER2 status, and would help to increase our understanding of this newly-proposed HER2 category in breast cancers.

## INTRODUCTION

The human epidermal growth factor receptor 2 (HER2) is an important biomarker for breast cancer prognosis and predictive efficacy of HER2-targeted therapy. According to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 testing guidelines, HER2 status should be routinely assessed by immunohistochemistry (IHC) and/or in-situ hybridization (ISH) in all newly-diagnosed breast cancers[1]. Based on results from early clinical trials which showed that only HER2-positive breast cancer patients benefited from the addition of trastuzumab to chemotherapy regimens[2–5], the evaluation of HER2 status in breast cancer has focused on separating HER2-positive cancers from HER2-negative cancers. This binary positive or negative paradigm of HER2 evaluation in breast cancers has been applied in routine clinical practice around the world.

Intriguingly, results from recent clinical trials have demonstrated significant clinical benefits from novel therapeutic compounds, particularly the new HER2-targeting antibody-drug conjugates (ADCs), in a subset of patients who have traditionally been considered as HER2 negative by IHC and ISH, but had low

levels of HER2 IHC expression[6,7]. A new concept of "HER2-low" breast cancer (breast cancers with HER2 IHC score of 1+, or HER2 IHC score of 2+ with a negative ISH result) has been proposed and applied in recent and ongoing clinical trials[5–13].

Based on the current proposed definition for HER2-low breast cancers, breast cancers with HER2 IHC score of 1+, or HER2 IHC score of 2+ with a negative ISH result will be the targeted populations for these novel HER2-targeting ADCs; however, this "HER2-low" category of breast cancers has rarely been studied and remains largely undefined. Therefore, the aims of this study were to investigate the incidence, HER2 IHC staining patterns, clinicopathologic features, and genomic profile of HER2-low breast cancers, in order to gain a better understanding of this newly-proposed HER2 category for breast cancers.

## MATERIALS AND METHODS

A retrospective search of the pathology laboratory information system (SoftPath IV) at the University of Rochester Medical Center (URMC) was performed to identify breast cancers diagnosed on core biopsy between

---

[1]Department of Pathology, University of Rochester Medical Center, Rochester, NY, USA. [2]Agendia Inc, Irvine, CA, USA. [3]These authors contributed equally: Huina Zhang, Hani Katerji. ✉email: Huina_Zhang@urmc.rochester.edu; David_Hicks@urmc.rochester.edu
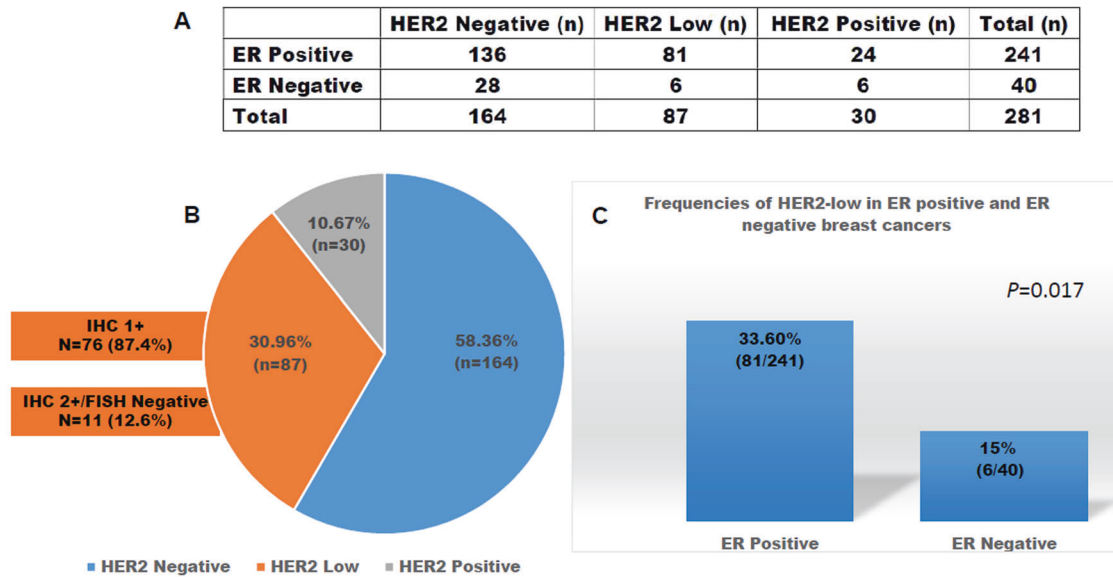
**Fig. 1 Incidence of HER2-low breast cancers. A** A table showing the case distribution in the study population; **B** A Pie chart showing the incidence of HER2-negative, HER2-low, and HER2-positive in the study population; **C** incidence of HER2-low breast cancers in estrogen receptor (ER)-positive and ER-negative breast cancers.

04/2020 and 12/2020. During this study period, all breast cancers diagnosed on core biopsy (n = 281) at our institution were sent for MammaPrint and BluePrint molecular testing to help triage patients for surgical vs systemic treatment, in response to the COVID19 Pandemic Breast Cancer Consortium Expert Opinion[14]. The study was approved by our institution's Institutional Review Board.

Clinicopathologic characteristics (age at diagnosis, size of tumor, pathologic tumor stage, pathologic lymph-node stage, clinical stage), histomorphologic features (histologic type, histologic grade, lymphovascular invasion), prognostic factors (estrogen receptor [ER], progesterone receptor [PR], HER2, and Ki-67) and genomic profile (MammaPrint risk of recurrence and BluePrint molecular subtypes) of all 281 cases were retrospectively collected. A 3-tier HER2 scoring system was used in this study: (1) HER2-negative (HER2 IHC score of 0+), (2) HER2-low (HER2 IHC score of 1+, or 2+ with a non-amplified ISH), and (3) HER2-positive (HER2 IHC score of 3+ and IHC score of 2+ with an amplified ISH).

HER2 IHC staining was performed by the Hercep TEST[TM], using an automated Dako 48 links Autostainer. HER2 fluorescent in-situ hybridization (FISH) was performed ([DAKO]—HER2 IQFISH pharmDx[TM]) on all equivocal HER2 IHC results, and the FISH results were used in lieu of the IHC to define the HER2 status for these cases, according to the most updated ASCO/CAP HER2 testing guidelines[1]. HER2 IHC stained slides were re-reviewed by at least two subspecialized breast pathologists (H.Z. and H.K., and also by D.G.H. for equivocal cases) to reach consensus on the HER2 scoring and HER2 staining pattern. HER2 IHC scoring of 0+ was defined as either no staining observed or incomplete membrane staining that is faint /barely perceptible within ≤10% of invasive tumor cells; HER2 IHC scoring of 1+ was defined as incomplete faint/barely perceptible membrane staining within >10% of invasive tumor cells; HER2 scoring of 2+ was defined as weak to moderate complete membrane staining observed in >10% of invasive tumor cells; and HER2 scoring of 3+ was defined as complete, intense circumferential membranous staining in >10% of invasive tumor cells[1]. HER2 staining patterns were recorded as either homogenous or heterogeneous with the heterogeneous pattern being further defined as either clustered, mosaic, or scattered. A HER2 homogenous staining pattern was defined as an overall evenly-distributed HER2 staining cells within the tumor. A HER2 heterogeneous staining pattern was defined as the presence of geographic differences in HER2 staining within the same tumor. The "clustered (regional)" pattern was defined as segregated populations of HER2-stained tumor cells and non-stained tumor cell populations. The "mosaic (intermixed)" pattern was defined as a tumor displaying HER2-stained cells comingled with non-stained tumor cells. The "scattered" pattern was defined as a tumor demonstrating isolated HER2-stained cells in a background of a non-stained tumor cell population. The status of ER (ERα [clone EP1], PR [clone

PgR1294]), and Ki-67 ([clone MIB-1]) were extracted from the pathology report.

For all 281 cases in this study, ten unstained slides at 5 microns interval sections were sent for MammaPrint testing (a 70-gene risk of recurrence assay) and Blueprint testing (an 80-gene molecular subtyping assay), both performed at Agendia, Inc (Agendia, Inc, Irvine, CA, USA)[15,16]. The MammaPrint results were reported by Agendia, Inc as low risk or high risk, and were further interpreted at URMC as MammaPrint Index (MPI) ultra-low risk (score +0.355 to +1.0), MPI low risk (score 0 to +0.355), MPI high risk 1 (H1, score 0.0 to −0.57), or MPI high-risk 2 (H2, score −0.57 to −1.0)[17,18]. The BluePrint results were reported by Agendia, Inc as luminal A, luminal B, HER2, or basal types.

For statistical analysis, a two-sample independent t-test and Fisher's exact test were used for numeric data and categorical data, respectively. A p value of <0.05 was considered statistically significant.

## RESULTS

Of the 281 consecutive breast cancers diagnosed on core biopsies at our institution during the study period, 58.4% (n = 164) were HER2-negative, 31% (n = 87) were HER2-low, and 10.6% (n = 30) were HER2-positve (Fig. 1). 34.7% (87/251) of patients who would be considered as HER2 negative by the current ASCO/CAP guidelines, were reclassified to HER2-low. Of the 87 patients reclassified to HER2-low breast cancers, 87.4% (76/87) had a HER2 IHC score of 1+, and 12.6% (11/87) had a HER2 IHC score of 2+. HER2-low cases accounted for 15% of the cases in the ER-negative group, and 33.6% of the cases in the ER-positive group (p = 0.017) (Fig. 1).

Of the 87 HER2-low cases, 76 (87.4%) cases were available for evaluation of HER2 staining pattern. 59.2% (n = 45) showed a homogenous pattern of HER2 staining, including 40 cases with scores of 1+ and 5 cases with score of 2+. 40.8% (n = 31) showed a heterogeneous HER2 staining pattern, including 34.2% with a clustered staining pattern (n = 26, all were an IHC score of 1+), and 6.6% with a mosaic staining pattern (n = 5, 4 cases with an IHC score of 1+ and 1 case with an IHC score of 2+) (Fig. 2). All 45 cases with homogenous HER2 staining pattern were ER-positive, and 44 of these 45 cases were luminal molecular types (one remaining case was HER2 molecular type). Twenty-four of 26 cases with HER2 clustered heterogeneous staining pattern were ER-positive, and 25 of these 26 cases were luminal molecular type

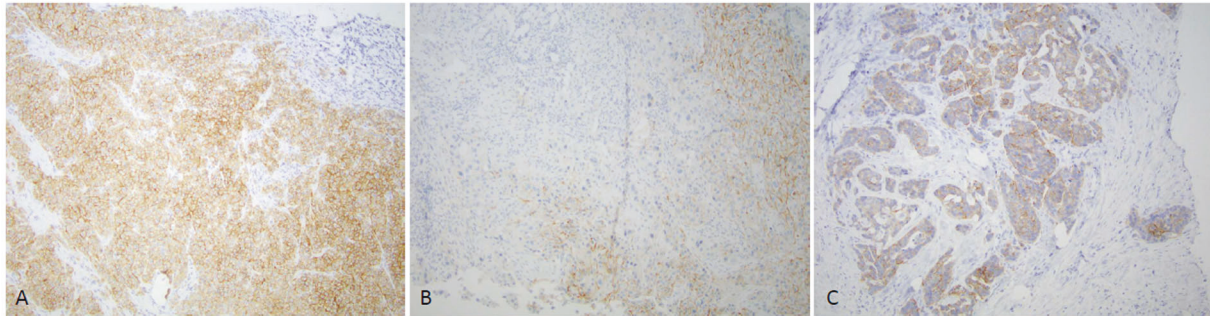| | Homogeneous staining | Heterogeneous staining | | |
|---|---|---|---|---|
| | | Clustered | Mosaic | Scattered |
| N (%) | 45 (59.2) | 26 (34.2) | 5 (6.6) | 0 |
| HER2 1+ (n,%) | 40 (88.9) | 26 (100) | 4 (80) | 0 |
| HER2 2+ (n,%) | 5 (11.1) | 0 | 1 (20) | 0 |



**Fig. 2  HER2 immunohistochemical staining patterns in HER2-low breast cancers. A** A HER2-low breast cancer with IHC score of 2+ and negative FISH (HER2/CEP17 ratio of 1.8 and average HER2 signal copy number per cell of 2.5, showing homogenous staining (×40); **B** a HER2-low breast cancer showing heterogeneous staining, clustered pattern (×40); **C** a HER2-low breast cancer showing heterogeneous staining, mosaic pattern (×40).

(one remaining case was basal molecular type). 3 of 5 cases with HER2 mosaic heterogeneous staining pattern were ER-positive and were luminal molecular type (two remaining cases were basal molecular type). When compared to the cases with homogenous staining pattern, the cases with mosaic heterogeneous pattern appeared to have more ER negativity and more likely to be basal molecular type (0% vs 40%, $p < 0.01$).

Table 1 lists the clinicopathologic features of HER2-low breast cancers. Briefly, the majority of HER2-low breast cancers were clinical stage I–II disease (79.0%), and 10.5% of cases being stage III disease, 6.6% of cases being stage IV disease, and 3.9% of cases being recurrent disease. 81.6% of HER2-low cancers were found in women aged ≥ 50 years, 81.6% had a ductal phenotype, 94.2% had histologic grades of 1 or 2, 93.1% were ER-positive, and 86.2% were PR positive. Genomic profiling results showed that 65.5% of HER2-low breast cancers had a luminal A molecular subtype, with a low risk for disease recurrence. 34.5% of cases had a high-risk for disease recurrence by MammaPrint testing, including 28.8% cases with a luminal B molecular subtype, 1.1% of cases with a HER2 molecular subtype, and 4.6% of cases with a basal molecular subtype (Table 2).

Table 3 illustrates the comparison of clinicopathologic parameters and molecular subtypes between HER2-low versus HER2-negative cases, and HER2-low versus HER2-positive cases. Compared to HER2-negative breast cancers, HER2-low breast cancers showed significantly lower Ki-67 ($p = 0.03$), higher ER positivity ($p = 0.01$), fewer cases with clinical stage II disease ($p = 0.01$), and were significantly less likely to be of the basal molecular subtype ($p < 0.01$). There was no significant difference in age, tumor size, histologic type, histologic grade, presence of lymphovascular invasion, and PR status. Compared to HER2-positive breast cancers, HER2-low breast cancers showed a significant tendency to occur in older women ($p < 0.01$), had significantly higher ER and PR positivity ($p = 0.03$ and $p < 0.01$), lower histologic grade ($p < 0.01$), lower Ki-67 ($p < 0.01$), fewer cases with clinical stage II disease ($p < 0.01$), and were significantly less likely to be of HER2 molecular subtype ($p < 0.01$).

In order to understand whether HER2-low breast cancers are distinctly different from HER2-negative cases, we further compared all of these clinicopathological variables between HER2-low and HER2-negative cases with adjustment of ER status. Interestingly, when adjusted for ER status, there was no significant difference in any of the examined variables between the HER2-low and HER2-negative groups (Table 4). However, the interpretation of results in the ER-negative group should be cautioned due to the limited case numbers.

In the HER2-low group, three patients received neoadjuvant chemotherapy. All three cases were histologic grade 3, ER-negative, PR-negative, and had a HER2 score of 2+. Genomic profiling with MammaPrint showed that all three were high-risk for disease recurrence. Genomic profiling with BluePrint revealed that two were basal subtypes, with the third being luminal B subtype. None of these three cases achieved pathologic complete response (pCR). In contrast, 4/14 (28.6%) HER2-negative patients and 7/12 (58.4%) HER2-positive patients achieved pCR (Table 4). While the response to neoadjuvant chemotherapy was poorer in HER2-low cases, when compared to HER2-negative and HER2-positive cases, the differences did not reach statistical significance (Table 3).

## DISCUSSION

The recent publications reporting a promising efficacy for novel HER2-targeted therapy with ADC in low-HER2 expressing breast cancers[6,7], have garnished increasing attention for the addition of a "HER2-low" category for breast cancer. In this study, we retrospectively investigated the incidence, HER2 IHC staining patterns, clinicopathologic features, and genomic profiles of HER2-low breast cancers, compared to HER2-negative and HER2-positive breast cancers.

The exact population incidence of HER2-low breast cancers is unknown, although it is estimated to be approximately 45–55% of breast cancers[11,13,19,20], based on studies using variable HER2 scoring criteria before the publication of 2013 and updated 2018 ASCO/CAP HER2 testing guidelines. After the new concept of "HER2-low" in breast cancer was introduced, two studies reported incidences of 31% and 51% for HER2-low breast cancer, based on data from The Cancer Genome Atlas and a clinical trial dataset, respectively[21,22]. Neither of these studies had a central

**Table 1.** The clinicopathologic parameters of HER2-low breast cancers.

| | |
|---|---|
| Age (Y) (mean, range) | 66.62 (37–88) |
| <50 Y (n, %) | 16 (18.4) |
| ≥50 Y (n, %) | 71 (81.6) |
| Tumor size (mm, mean, range) | 21.5 (3–153) |
| Histologic type | |
| Ductal (n, %) | 71 (81.6) |
| Lobular (n, %) | 15 (17.2) |
| Mixed (n, %) | 1 (1.2) |
| Histologic grade | |
| 1 (n,%) | 43 (49.4) |
| 2 (n,%) | 39 (44.8) |
| 3 (n,%) | 5 (5.8) |
| pT stage | |
| 1 (n,%) | 42 (75) |
| 2 (n,%) | 8 (14.3) |
| 3 (n,%) | 6 (10.7) |
| Unknown/Neoadjuvant | 31 |
| pN stage | |
| 0 (n, %) | 36 (83.7) |
| 1 (n, %) | 6 (14.0) |
| 2 (n, %) | 1 (2.3) |
| 3 (n, %) | 0 (0) |
| Unknown/Neoadjuvant | 44 |
| Clinical stage | |
| I (n, %) | 50 (65.8) |
| II (n, %) | 10 (13.2) |
| III (n, %) | 8 (10.5) |
| IV (n, %) | 5 (6.6) |
| Recurrence (n, %) | 3 (3.9) |
| Unknown | 11 |
| Lymphovascular invasion | |
| Present (n, %) | 6 (11.5) |
| Absent (n, %) | 46 (88.5) |
| Unknown | 35 |
| ER | |
| Positive (n, %) | 81 (93.1) |
| Negative (n, %) | 6 (6.9) |
| PR | |
| Positive (n, %) | 75 (86.2) |
| Negative (n, %) | 12 (13.8) |
| Ki-67 (%) (mean, range) | 12.8 (1–60) |

ER estrogen receptor, PR progesterone receptor.

**Table 2.** The genomic profiles of HER2-low breast cancer by MammaPrint and BluePrint analysis.

| | Case numbers | % |
|---|---|---|
| MPI by MammaPrint | | |
| Ultra-low risk (Score +0.355 to +1.0) | 25 | 28.7 |
| Low risk (Score 0 to +0.355) | 32 | 36.8 |
| High risk-H1 (Score 0.0 to −0.57) | 27 | 31.0 |
| High risk-H2 (Score −0.57 to −1.0) | 3 | 3.5 |
| Molecular subtype by BluePrint | | |
| Luminal A | 57 | 65.5 |
| Luminal B | 25 | 28.8 |
| HER2 | 1 | 1.1 |
| Basal[a] | 4 | 4.6 |

MPI MammaPrint Index.
[a]All the cases with basal molecular types were ER negative/PR negative.

confirmation of HER2 status. In our study, we re-evaluated 281 consecutive breast cancer cases diagnosed at our institution, and HER2-low breast cancers were confirmed, with consensus, in 31% of our study population. Under the current definition, identification of HER2-low breast cancer predominantly relies on the HER2 status tested by a semi-quantitative IHC assay, and these assay interpretations may be impacted by a number of pre-analytic, analytic, and post-analytic factors[7,23–25]. For example, Lambein et al. found that central reassessment of breast cancers scored as IHC negative after local laboratory testing resulted in an important shift (75% of cases) of a HER2 score of 0 toward a HER2 score of 1+[24]. A preliminary study by Scott et al. demonstrated that the VENTANA 4B5 antibody clone identified a higher proportion of HER2-low cases than HercepTest (27.4% vs 9.2%) in a subset analysis of 500 cases[26]. We have also observed an inter-observer and inter-antibody variation in the evaluation of HER2 IHC, particularly in cases with 0 to 1+ HER2 staining[27]. The variable incidences of HER2-low breast cancers among studies demonstrate the challenges of using IHC as the primary assay in identifying HER2-low breast cancer. Until a more consistently reproducible methodology is available, careful evaluation of HER2 IHC with consensus between observers and/or additional training, may be necessary for the accurate evaluation of HER2-low breast cancers, in order to better stratify patients into groups who are more likely to benefit from the newer ADC therapeutic agents used to treat HER2-low breast cancers.

The clinical benefits of the newly-developed ADCs, including trastuzumab duocarmazine (SYD-985) and trastuzumab deruxtecan (T-DXd), in the HER2-low breast cancers, are likely achieved by the so-called "bystander-killing" mechanisms. This is distinctly different from the more traditional HER2-targeted agents, which either direct inhibition of HER2 dimerization or the blockade of down-stream signaling[6,7,11]. In the "bystander-killing" model, HER2 molecules on the tumor cell surface primarily function as a means for delivering antibody-conjugated drugs. The pre-clinical in vitro and in vivo studies have demonstrated that T-DXd induces a potent "bystander-killing" effect on cells in close proximity to targeted HER2-expressing tumor cells, regardless of their HER2 status, through the transfer of released payload from the antigen-expressing cells to the neighboring antigen-negative cells[11,28]. In vitro studies have demonstrated that threshold densities of 50,000-200,000 HER2 receptor molecules on the cell membrane, which correspond to HER2 IHC scores of 1+ to 2+[29,30], are necessary for efficient binding of HER2-targeted model ADCs and triggering the ADC internalization into the tumor cells[31,32]. Therefore, it is reasonable to hypothesize that the homogenous and mosaic patterns of HER2 staining may respond better to these newer ADCs than those of "clustered" and "scattered" patterns of staining in the HER2-low breast cancers. In the current study, we reported specific HER2 staining patterns for the first time in HER2-low breast cancers. 65.8% of HER2-low breast cancer cases showed either a homogenous or mosaic patterns, indicating that more than 50% of HER2-low breast cancers may potentially respond to these ADCs. Larger studies, particularly studies using clinical trial data, are necessary to validate this finding.

Earlier retrospective studies on low HER2-expressing breast cancers have been focused on the difference between HER2 2+

**Table 3.** Comparison of clinicopathologic parameters and molecular subtypes between HER2-low and HER2-negative/positive breast cancers.

| | HER2 low (n = 87) | HER2 negative (n = 164) | HER2 positive (n = 30) | P value (HER2 low vs negative) | P value (HER2 low vs positive) |
|---|---|---|---|---|---|
| Age (year, mean) | 66.62 | 64.38 | 56.73 | 0.22 | <0.01 |
| Tumor size (mm, mean) | 21.49 | 19.29 | 26.67 | 0.49 | 0.38 |
| Histologic type | | | | | |
| Ductal (n,%) | 71 (81.6) | 139 (84.8) | 27 (90) | 0.52 | 0.39 |
| Lobular (n,%) | 15 (17.2) | 24 (14.6) | 3 (10) | 0.59 | 0.56 |
| Mixed (n,%) | 1 (1.2) | 1(0.6) | 0 | 1.00 | 1.00 |
| Histologic grade | | | | | |
| 1 (n,%) | 43 (49.4) | 64 (39.0) | 1 (3.3) | 0.14 | <0.01 |
| 2 (n,%) | 39 (44.8) | 81 (49.4) | 15 (50.0) | 0.51 | 0.67 |
| 3 (n,%) | 5 (5.8) | 19 (11.6) | 14 (46.7) | 0.18 | <0.01 |
| LVI present (%) | 11.5 (6/52) | 11.6 (14/121) | 15.8 (3/19) | 1.0 | 0.693 |
| Clinical stage | | | | | |
| I (n,%) | 50 (65.8) | 87 (61.3) | 13 (44.8) | 0.51 | 0.074 |
| II (n,%) | 10 (13.2) | 40 (28.2) | 14 (48.3) | 0.01 | <0.01 |
| III (n,%) | 8 (10.5) | 7 (4.9) | 2 (6.9) | 0.16 | 0.72 |
| IV (n,%) | 5 (6.6) | 3 (2.1) | 0 | 0.13 | 0.32 |
| Recurrence (n,%) | 3 (3.9) | 5 (3.5) | 0 | 1.00 | 0.56 |
| Unknown | 11 | 22 | 1 | NA | NA |
| ER positivity (%) | 93.1% (81/87) | 82.9% (136/164) | 80% (24/30) | 0.01 | 0.03 |
| PR positivity (%) | 85.5% (74/87) | 76.8% (126/164) | 50% (15/30) | 0.09 | <0.01 |
| Ki-67 (%) | 12.81 | 18.29 | 32.3 | 0.03 | <0.01 |
| Molecular subtype | | | | | |
| Luminal A (n, %) | 57 (65.5) | 105 (64.0) | 1 (3.3) | 0.89 | <0.01 |
| Luminal B (n, %) | 25 (28.8) | 31 (18.9) | 9 (30) | 0.08 | 1.00 |
| HER2 (n, %) | 1 (1.1) | 0 | 19 (63.4) | 0.35 | <0.01 |
| Basal (n, %) | 4 (4.6) | 28 (17.1) | 1 (2.2) | <0.01 | 1.00 |
| Response to NAC | | | | | |
| pCR (n,%) | 0 | 4 (28.6) | 7 (58.4) | 0.29 | 0.2 |
| RCB I (n, %) | 0 | 2 (14.3) | 1 (8.3) | 1.00 | 1.00 |
| RCB II–III (n,%) | 3 (100) | 8 (57.1) | 4 (33.3) | 0.52 | 0.08 |

*ER* estrogen receptor, *LVI* lymphovascular invasion, *NAC* neoadjuvant chemotherapy, *pCR* pathologic complete response, *PR* progesterone receptor, *RCB* residual cancer burden.

with negative ISH and HER2 0/1+[20,33]. Due to the increased interest in the HER2-low breast cancers, it is important to better characterize the clinicopathologic features of HER2-low breast cancers according to the proposed definition. Recently, Schettini et al.[25] reported the clinical, pathological and molecular features of 3689 HER2-low breast cancers, based on a pooled-dataset analysis from multiple clinical trials. Their results showed that HER2-low breast cancers were more frequently found to have hormone receptor-positive expression (88.2%) with either luminal A or luminal B subtypes (79.6%), were more frequent in older and male patients, and showed more axillary lymph-node involvement compared to HER2 0 disease. Denkert and colleagues[22] performed a pooled analysis of 2310 patients from four prospective neoadjuvant clinical trials. In that study, HER2-low breast cancers tended to show higher hormone receptor expression, lower cell proliferation, lower tumor grade, low pCR to the neoadjuvant chemotherapy, and significantly longer survival. The major limitations of these two studies include the patient population and a lack of central review of HER2 status. In both studies, patients were pooled from different clinical trial database and from different studies, with different original purposes and different inclusion/exclusion criteria. In addition, both studies

tended to have patients with breast cancers with more aggressive features or higher clinical stages. In the current study, we analyzed 281 consecutive, real-world cases with a consensus confirmation of HER2 status. Our results were essentially consistent with those from earlier studies and reinforced that the majority of HER2-low breast cancers are grades 1 or 2, early-stage, hormonal receptor positive, have a HER2 IHC score of 1+, and are less likely to achieve pCR to neoadjuvant chemotherapy.

Currently, the biology of HER2-low breast cancers is largely unknown. Genomic profile analysis can provide insights on the molecular subtypes and prognostic gene signatures of HER2-low breast cancers. Two earlier studies on molecular profiling of HER2-low breast cancers were both done using the PAM50 assay[21,25]. The current study is the first to examine the molecular profile of HER2-low breast cancer using the MammaPrint and BluePrint genomic assays. PAM50 is a qRT-PCR based, 50-gene molecular profile assay that classifies patients into luminal A-, luminal B-, HER2-enriched- and basal-like subtypes. An 18 gene subset of the 50-gene molecular profile is used to calculate a proliferation score and determine the risk of recurrence in combination with the molecular profile and the tumor size. The MammaPrint assay is a microarray-based technology that measures the expression of 70

H. Zhang et al.

**Table 4.** Comparison of clinicopathologic parameters and molecular subtypes in ER-positive and ER-negative breast cancers between HER2-low and HER2-negative groups.

| | ER positive | | | ER negative | | |
|---|---|---|---|---|---|---|
| | HER2 low (*n* = 81) | HER2 negative (*n* = 136) | *P* value | HER2 low (*n* = 6) | HER2 negative (*n* = 28) | *P* value |
| Age (year, mean) | 66.76 | 65.25 | 0.41 | 64.66 | 60.2 | 0.54 |
| Tumor size (mm, mean) | 21.07 | 19.00 | 0.55 | Not performed[a] | | |
| Histologic type | | | | | | |
| Ductal | 65 (80.2) | 112 (82.4) | 0.72 | 6 (100) | 27 (96.4) | 1.00 |
| Lobular | 15 (18.5) | 23 (16.9) | 0.85 | 0 | 1 (3.6) | 1.00 |
| Mixed | 1 (1.2) | 1 (0.7) | 1.00 | 0 | 0 | NA |
| Histologic grade | | | | | | |
| 1 | 40 (49.4) | 64 (47.0) | 0.78 | 1 (16.7) | 0 | 0.18 |
| 2 | 38 (46.9) | 67 (49.3) | 0.78 | 3 (50.0) | 14 (50.0) | 1.00 |
| 3 | 3 (3.7) | 5 (3.7) | 1.00 | 2 (33.3) | 14 (50.0) | 0.26 |
| LVI present (%) | 8 (4/50) | 12.1 (12/99) | 0.44 | Not performed[a] | | |
| Clinical stage | | | | | | |
| I–III | 60 (89.5) | 114 (95.8) | 0.59 | 4 (80.0) | 20 (87.0) | 0.30 |
| IV | 4 (6.0) | 2 (1.7) | 0.19 | 1 (20.0) | 1 (4.3) | 0.33 |
| Recurrence | 3 (4.5) | 3 (2.5) | 0.35 | 0 | 2 (8.7) | 1.00 |
| Unknown | 14 | 17 | NA | 1 | 5 | NA |
| PR positivity (%) | 91.3 (74/81) | 94.8 (129/136) | 0.91 | 0 | 0 | NA |
| Ki-67 (%) | 10.03 | 11.26 | 0.38 | 38.3 | 51.9 | 0.29 |
| Molecular subtype | | | | | | |
| Luminal A (*n*, %) | 57 (70.4) | 103 (75.7) | 0.43 | 0 | 2 (7.1) | 1.00 |
| Luminal B (*n*, %) | 23 (28.4) | 30 (22.1) | 0.33 | 2 (33.3) | 1 (3.6) | 0.074 |
| HER2 (*n*, %) | 1 (1.2) | 0 (2.2) | 0.37 | 0 | 0 | NA |
| Basal (*n*, %) | 0 | 3[b] (2.2) | 0.30 | 4 (66.7) | 25 (89.3) | 0.67 |

*ER* estrogen receptor, *LVI* lymphovascular invasion, *NA* Not available/applicable, *PR* progesterone receptor.
[a]Data analysis not performed due to most of patients received neoadjuvant chemotherapy.
[b]Two cases were ER-low positive/PR negative; One case was ER positive (~30% with moderate staining)/PR negative.

genes that are involved in cell cycle and proliferation, invasion and metastasis, angiogenesis, and signal transduction. BluePrint is another microarray-based, 80-gene profile assay that determines the intrinsic breast cancer molecular subtypes by measuring the similarity of the tested tumor to that of luminal-type (58 genes), basal-type (28 genes), and HER2-type (4 genes). When combined with MammaPrint results, BluePrint further classifies luminal-type tumors into luminal A (low risk) or luminal B (high risk). Although there are only 9 genes in common between PAM50 and BluePrint[16], it has been demonstrated that the classification of breast tumors into luminal, HER2, and basal subtypes by PAM50 or BluePrint has moderate to great agreement[16,34]. Using the PAM50 assay, both Schettini et al. and Agostinetto et al.[21,25] demonstrated that HER2-low breast cancers are composed of a heterogeneous group of breast cancers including luminal A (50.8–56.9%), luminal B (22.8–28.8%), HER2-enriched (3.5–3.6%), and basal-like (13.3–17.7%). Our BluePrint/MammaPrint results on HER2-low breast cancers also showed similar distributions of these molecular subtypes: 65.5% of the luminal A subtype, 28.8% of the luminal B subtype, 1.1% of the HER2 subtype, and 4.6% of the basal subtype. These molecular profiling results indicate that the majority of HER2-low breast cancers (79–94%) are luminal gene-driven, although with low-HER2 expression on IHC. In ER+/HER2-low breast cancers, approximately 50-60% of cancers are luminal A type with a biologically lower risk for recurrence, and approximately 25–30% are luminal B type with a comparably biologically higher risk for recurrence. It would be interesting to investigate the differences between low-risk and high-risk ER+/HER2-low

breast cancers in the whole transcriptome level, in order to better understand HER2-low breast cancers. Interestingly, HER2-low breast cancers were also classified into the HER2 molecular subtype in these studies, although very rare (1.1–3.6%). Schettini et al.[25] found *ERBB2* gene expression was significantly higher in HER2-low/hormone receptor (HR)+ tumors than that of HER2 0/HR+ tumors with the highest amount observed in HER2 IHC 2+ tumors, followed by 1+ and 0. HR-negative/HER2-low tumors had statistically significantly higher levels of *ERBB2* compared to HER2 0 tumors; however, there was no statistically significantly difference across the three HER2 IHC groups. Agostinetto et al.[21] also demonstrated that levels of *ERBB2* mRNA expression were higher in HER2-low tumors compared to HER2-negative tumors, with significantly higher expression in HER2-low/HR-positive tumors compared to HER2-low/HR-negative tumors. Since HER2 molecular type is not defined by the single *HER2* gene expression, it is unclear whether this is due to increased gene expression, or as a result of an alternate mechanism other than the amplification by which the HER2 pathway is known to be activated.

Additionally, it is still unclear whether HER2-low breast cancers represent a biologically distinct category from HER2-negative breast cancers. The findings from Schettini et al.[25] and Denkert et al.[22] may indicate HER2-low breast cancers are biologically distinct from HER2-negative breast cancers. In the study by Schettini et al.[24], PAM50 intrinsic subtypes and 34 of 55 genes (61.8%) were differentially distributed between HER2-low and HER2-negative breast cancers, and this significant difference remained in the HR-positive disease population, but not in the

HR-negative disease population. On the contrary, Agostinetto et al.[21] reported that PAM50 intrinsic subtypes significantly differed between HER2-negative and HER2-low in the HR-negative tumors, while the difference was not statistically significant when comparing HER2-negative/HR-positive vs HER2-low/HR-positive tumors. Overall, the intrinsic molecular subtype distributions in the HER2-low breast cancers among HR-positive breast cancers in our study were more similar to the study of Agostinetto et al.[21], supporting that HER2-low/HR-positive breast cancers are biologically similar to HER2-negative/HR-positive breast cancers. Our results did demonstrate that HER2-low breast cancers had some distinct differences compared to HER2-negative breast cancers, particularly with regards to hormone receptor status, tumor proliferation activity, intrinsic molecular subtypes and response to neoadjuvant chemotherapy; however, when further adjusted for ER status, these differences did not reach statistical significance. It did appear that any significant differences observed between HER2-low and HER2-negative groups were mainly associated with the ER-negative breast cancers; however, in our study ER-negative, HER2-low breast cancers only accounted for approximately 7% of the study population, and due to the small case numbers included for analysis in the ER-negative cases (HER2-low, $n = 6$; HER2-negative, $n = 28$), the significance of this observation cannot be confirmed. Our findings, when examined alongside the findings of Schettini et al.[25] and Agostinetto et al.[21], emphasize the importance of considering the percentage and intensity of ER status in future studies investigating biological comparisons between HER2-negative and HER2-low breast cancers.

There are some limitations in this study. First, the retrospective nature of this study has its associated biases. Second, a relatively small number of cases were included in this cohort, particularly with respect to HER2-low/ER-negative cases, which limits the significance of any conclusions in this group. We recognize these limitations and look forward to a prospective study with a larger cohort in the future.

Our study is different from previous reports on HER2-low breast cancers, being by far the first study to investigate the incidence, HER2 IHC staining patterns, clinicopathologic features, and genomic profile of HER2-low breast cancers deriving from the consecutive, single-institution based cases after re-evaluation and consensus confirmation of HER2 status, using the MammaPrint and BluePrint genomic assays. Our results provide valuable baseline characteristics of HER2-low breast cancers, and would add value to the existing limited body of knowledge regarding HER2-low breast cancers, and will hopefully help to increase our understanding of this newly-proposed HER2 category in breast cancers. Further investigations are needed on HER2-low-related topics, including additional studies to further validate the findings from this single-institution study.

## DATA AVAILABILITY
All data generated or analyzed in this study are included in this article.

## REFERENCES
1. 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. *Arch. Pathol. Lab. Med.* **142**, 1364–1382 (2018).
2. Pegram, M. D. et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.* **16**, 2659–2671 (1998).
3. Cobleigh, M. A. et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* **17**, 2639–2648 (1999).
4. Robert, N. et al. Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2-overexpressing metastatic breast cancer. *J. Clin. Oncol.* **24**, 2786–2792 (2006).
5. Fehrenbacher, L. et al. NSABP B-47/NRG oncology phase III randomized trial comparing adjuvant chemotherapy with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by FISH and with IHC 1+ or 2. *J. Clin. Oncol.* **38**, 444–453 (2020).
6. Banerji, U. et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol.* **20**, 1124–1135 (2019).
7. Modi, S. et al. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low-expressing advanced breast cancer: results from a phase Ib study. *J. Clin. Oncol.* **38**, 1887–1896 (2020).
8. Chick, R. C. et al. Subgroup analysis of nelipepimut-S plus GM-CSF combined with trastuzumab versus trastuzumab alone to prevent recurrences in patients with high-risk, HER2 low-expressing breast cancer. *Clin. Immunol.* **225**, 108679 (2021).
9. Mittendorf, E. A. et al. Efficacy and safety analysis of Nelipepimut-S vaccine to prevent breast cancer recurrence: a randomized, multicenter, Phase III clinical trial. *Clin. Cancer Res.* **25**, 4248–4254 (2019).
10. Schneeweiss, A. et al. Phase Ib study evaluating safety and clinical activity of the anti-HER3 antibody lumretuzumab combined with the anti-HER2 antibody pertuzumab and paclitaxel in HER3-positive, HER2-low metastatic breast cancer. *Invest. New Drugs* **36**, 848–859 (2018).
11. Zhang, H., Katerji, H., Turner, B. M. & Hicks, D. G. HER2-low breast cancers: new opportunities and challenges. *Am. J. Clin. Pathol.* https://doi.org/10.1093/ajcp/aqab117 (2021).
12. Marchiò, C. et al. Evolving concepts in HER2 evaluation in breast cancer: heterogeneity, HER2-low carcinomas and beyond. *Semin. Cancer Biol.* S1044-579X (20)30049-3 (2020).
13. Tarantino, P. et al. HER2-low breast cancer: pathological and clinical landscape. *J. Clin. Oncol.* **38**, 1951–1962 (2020).
14. Dietz, J. R. et al. Recommendations for prioritization, treatment, and triage of breast cancer patients during the COVID-19 pandemic. the COVID-19 pandemic breast cancer consortium. *Breast Cancer Res. Treat.* **181**, 487–497 (2020).
15. Cardoso, F. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. *N. Engl. J. Med.* **375**, 717–729 (2016).
16. Krijgsman, O. et al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. *Breast Cancer Res. Treat.* **133**, 37–47 (2012).
17. Esserman, L. J. et al. Use of molecular tools to identify patients with indolent breast cancers with ultralow risk over 2 decades. *JAMA Oncol.* **3**, 1503–1510 (2017).
18. Rugo, H. S. et al. Adaptive randomization of veliparib–carboplatin treatment in breast cancer. *N. Engl. J. Med.* **375**, 23–34 (2016).
19. Schalper, K. A., Kumar, S., Hui, P., Rimm, D. L. & Gershkovich, P. A retrospective population- based comparison of HER2 immunohistochemistry and fluorescence in situ hybridization in breast carcinomas: impact of 2007 American Society of Clinical Oncology/ College of American Pathologists Criteria. *Arch. Pathol. Lab. Med.* **138**, 213–219 (2014).
20. Rossi, V. et al. Moderate immunohistochemical expression of HER-2 (2+) without HER-2 gene amplification is a negative prognostic factor in early breast cancer. *Oncologist* **17**, 1418–1425 (2012).
21. Agostinetto, E. et al. HER2-low breast cancer: molecular characteristics and prognosis. *Cancers* **13**, 2824 (2021).
22. 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**, 1151–1161 (2021).
23. Goldstein, N. S., Hewitt, S. M., Taylor, C. R., Yaziji, H. & Hicks, D. G., Members of Ad-Hoc Committee On Immunohistochemistry Standardization. Recommendations for improved standardization of immunohistochemistry. *Appl. Immunohistochem. Mol. Morphol.* **15**, 124–133 (2007).
24. Lambein, K. et al. Distinguishing score 0 from score 1+ in HER2 immunohistochemistry-negative breast cancer: clinical and pathobiological relevance. *Am. J. Clin. Pathol.* **140**, 561–566 (2013).
25. Schettini, F. et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer* **7**, 1 (2021).
26. Scott, M., Vandenberghe, M., Scorer, P., Boothman, A. M. & Barker, C. Prevalence of HER2 low in breast cancer subtypes using the VENTANA anti-HER2/neu (4B5) assay. American Society of Clinical Oncology annual meeting 2021 Abstract. *J. Clin. Oncol.* **39** (Suppl 15;abstr 1021) (2021).
27. Zhang, H., Wang, X., Katerji, H., Turner, B. M. & Hicks D. G. Identifying HER2-low breast cancers by immunohistochemistry: inter-observer and inter-antibody reproducibility. United States and Canadian Academy of Pathology 111th annual meeting Abstract. *Mod. Pathol.* (Accepted as meeting abstract) (2022).
28. Ogitani, Y., Hagihara, K., Oitate, M., Naito, H. & Agatsuma, T. Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2

antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. *Cancer Sci.* **107**, 1039–1046 (2016).

29. Ross, J. S. et al. The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. *Oncologist* **8**, 307–325 (2003).

30. Onsum, M. D. et al. Single-cell quantitative HER2 measurement identifies heterogeneity and distinct subgroups within traditionally defined HER2-positive patients. *Am. J. Pathol.* **183**, 1446–1460 (2013).

31. Sharma, S., Li, Z., Bussing, D. & Shah, D. K. Evaluation of quantitative relationship between target expression and antibody-drug conjugate exposure inside cancer cells. *Drug Metab. Dispos.* **48**, 368–377 (2020).

32. Hendriks, B. S. et al. Impact of tumor HER2/ERBB2 expression level on HER2-targeted liposomal doxorubicin-mediated drug delivery: multiple low-affinity interactions lead to a threshold effect. *Mol. Cancer Ther.* **12**, 1816–1828 (2013).

33. Eggemann, H. et al. Moderate HER2 expression as a prognostic factor in hormone receptor positive breast cancer. *Endocr. Relat. Cancer* **22**, 725–733 (2015).

34. Bartlett, J. M. et al. Comparing breast cancer multiparameter tests in the OPTIMA prelim trial: no test is more equal than the others. *J. Natl. Cancer Inst.* **108**, djw050 (2016).

## AUTHOR CONTRIBUTIONS

H.Z. and D.G.H. performed study concept and design; H.Z. and H.K. performed development of methodology and data collection. H.Z., H.K., B.M.T., W.A. and D.G.H. performed writing, review, and revision of the paper; H.Z. and B.M.T. provided acquisition, analysis, and interpretation of data, and statistical analysis. All authors read and approved the final paper.

## COMPETING INTERESTS

W.A. is an employee of Agendia Inc. D.G.H. serves on the advisory board of AstraZeneca. All other authors declare no competing interests.

## ETHICAL APPROVAL

This study was approved by the Institutional Review Board.

## ADDITIONAL INFORMATION

**Correspondence** and requests for materials should be addressed to Huina Zhang or David G. Hicks.

**Reprints and permission information** is available at http://www.nature.com/reprints

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.