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Clinicopathologic characterization of hormone-receptor positive, human epidermal growth factor receptor 2 Null, Ultralow, and Low breast carcinoma in the metastatic setting

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ABSTRACT

HER2-directed therapy landscape is rapidly evolving. Patients with hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2) Low and HER2 Ultralow demonstrate clinically meaningful progression-free survival improvement with HER2-directed antibody drug conjugates. Herein, we assess rates of hormone receptor-positive (HR+), HER2 Null, HER2 Ultralow, and HER2 Low metastatic breast carcinoma (MBC) and review clinical and pathologic aspects of primary tumors. We retrospectively reviewed all patients diagnosed with HR+/HER2- MBC metastatic breast carcinoma between 01/2023-12/2023 and characterized patients with HER2 Low, Ultralow, and Null results. 99 samples from 95 patients showed HR+/HER2- MBC. Seventy (70.7%) patients had HR+/HER2 Low MBC, 21 (21.2%) had HR+/HER2 Ultralow MBC, and 8 (8.1%) had HR+/HER2 Null MBC. HR and HER2 status of primary breast carcinoma were available in 56 (56.6%) of 99 samples. Primary and metastatic breast carcinoma samples shared the same HER2 status in 39 (69.6%) of 56 cases. Two (4.1%) of HER2 Low and 1 (33.3%) of HER2 Ultralow primary breast carcinoma cases showed shift to HER2 Null in the metastatic setting, and 3 (75%) of HER2 Null primary breast carcinoma cases showed shift to HER2 Ultralow or HER2 Low. Our one-year single-institution retrospective study shows majority of MBC are HR+/HER2 Low, followed by HR+/HER2 Ultralow. While majority of primary and metastatic breast carcinoma samples share similar HER2 status, patients may show change in HER2 status on metastatic tumor samples and repeat biomarker testing on metastatic samples may meaningfully guide HER2-directed antibody drug conjugate therapy.

1. Introduction

The landscape of HER2-directed therapy is evolving at a rapid rate. The DESTINY-Breast04 and DESTINY-Breast06 trials showed patients with hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2) Low [Immunohistochemistry (IHC) 1+ or 2+ without amplification by *in situ* hybridization] and HER2 Ultralow advanced (IHC 0 with membranous staining in <10%) demonstrate statistically significant and clinically meaningful progression-free survival improvement when treated with HER2-directed antibody drug conjugates (e.g. trastuzumab deruxtecan) [1,2]. Currently, the U.S. Food and Drug Administration has approved HER2-directed antibody drug conjugates for the treatment of unresectable and metastatic breast carcinoma in multiple settings, including HR-positive, HER2 Low or Ultralow disease, as well as HR-negative, HER2 Low and HER2 Positive disease. Despite this, studies are limited on the clinical and pathological features of patients with HR-positive/HER2 Ultralow and Low metastatic breast carcinoma [3–5]. Herein, we provide the prevalence of HR-positive/HER2 Low, HR-positive/HER2 Ultralow, and HR-positive/HER2 Null rates in metastatic breast carcinoma and characterize eligible patients and their tumors in this one-year retrospective review from a single, large, quaternary care academic medical center.

2. Methods

2.1. Clinicopathologic data

This study was approved by the institutional review board (#19–1257). Pathology archives of the Robert J Tomsich Pathology and Laboratory Medicine Institute at Cleveland Clinic (Cleveland, Ohio)

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from January 1, 2023 to December 31, 2023 were searched for the diagnosis of metastatic breast carcinoma. Clinical and pathologic variables, including age at diagnosis of metastasis, sex, date of primary and metastatic carcinoma diagnoses, site of metastasis, primary tumor histology, primary and metastatic tumor hormone receptor [estrogen receptor (ER) and progesterone receptor (PR)] status, and primary and metastatic tumor HER2 immunohistochemistry and/or fluorescence *in situ* hybridization (FISH) results, adjuvant systemic treatment regimen, and clinical follow-up, were recorded for patients with metastatic breast carcinoma samples showing HR-positive/HER2 Low, HR-positive/HER2 Ultralow, and HR-positive/HER2 Null results.

2.2. ER, PR, and HER2 staining and scoring

All primary and metastatic tissue samples were stained in our institution laboratory using the protocols detailed herein.

For ER staining (CONFIRM ER SP1, Ventana Medical Systems, Tucson, AZ, USA), epitope retrieval was performed by incubating with CC1 buffer for 64 min. The tissue was incubated with the predilute antibody for 16 min at 36 °C and staining revealed in brown using the I-View DAB detection kit (Ventana Medical Systems). For PR staining (CONFIRM PR SP1, Ventana Medical Systems), epitope retrieval was performed by incubating with CC1 buffer for 64 min. The tissue was incubated with the predilute antibody for 16 min at 36 °C and staining revealed in brown using the I-View DAB detection kit (Ventana Medical Systems). For HER2 staining (PATHWAY HER2 4B5, Ventana Medical Systems), epitope retrieval was performed by incubating with CC1 buffer for 36 min. The tissue was incubated with the predilute antibody for 12 min at 36 °C and staining revealed in brown using the I-View DAB detection kit (Ventana Medical Systems). HR-positive disease was considered immunoreactive for ER and/or PR in greater than or equal to 1% of tumor cell nuclei. HER2 IHC and FISH were originally reported in accordance with contemporaneous American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) breast cancer guideline recommendations [6–10].

All cases with equivocal HER2 IHC (2+) results underwent FISH testing. For these cases, deparaffinized tissue sections were analyzed used a *HER2* dual-probe FISH assay (PathVysion *HER2* DNA Probe Kit, Vysis, Abbott Molecular, Des Plaines, IL, USA) as the primary methodology. Results were classified as negative, equivocal, or positive according to the contemporaneous ASCO/CAP guidelines, where applicable [11–14]. Cases with equivocal FISH results, per the relevant guideline at the time [11,12], underwent additional FISH testing to further define the HER2 status. This included FISH testing with an alternate chromosome 17 probe (*D17S122*) and/or reflex IHC testing. For equivocal FISH cases undergoing alternate probe, the *HER2: D17S122* (Empire Genomics, Buffalo, NY, USA) Probe Set was used, as previously described in Donaldson et al. [15].

ER and PR IHC testing and HER2 IHC and FISH testing are validated for formalin-fixed, paraffin-embedded tissue and for tissues that are decalcified with ethylenediaminetetraacetic acid (EDTA) prior to processing. All tissue samples were placed in 10% neutral buffered formalin within 60 min and fixed for 6–72 h prior to processing. Tissue samples containing bone were processed routinely or decalcified using EDTA prior to processing.

Hematoxylin and eosin (H&E)-stained and ER, PR, and HER2 IHC slides of all primary and metastatic cases and their scores were initially reviewed by a single expert breast pathologist (R.S.H.). Cases scored as HER2 IHC 0 cases were subsequently reevaluated through joint assessment by two expert breast pathologists (R.S.H. and P.J.M.) and further classified as HER2 Ultralow or HER2 Null. HER2 Low was defined as HER2 IHC 1+ or 2+/FISH not-amplified, HER2 Ultralow was defined as incomplete, faint/barely perceptible, and membranous staining in less than or equal to 10% of tumor cells, and HER2 Null was defined as absence of any membranous staining in tumor cells [2,16]. Patients without ER, PR, and HER2 testing performed on the metastatic tumor sample were excluded.

2.3. Statistical analysis

Statistical analysis was performed using RStudio Version 2024.9.1.394 (PBC, Boston, MA, USA) with the χ^2 test, Fisher exact test, one-way analysis of variance, and multinomial logistic regression, where applicable. Statistical significance of predictor variables was assessed using Wald test, with two-tailed p-values computed from the z-statistics derived from the model coefficients and standard errors. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Clinicopathologic characteristics

During the study period, 99 metastatic breast carcinoma specimens were obtained from 95 patients at our institution. Clinical and pathologic features for this cohort are summarized in Table 1. All patients were women, and median patient age was 69 years (mean, 67 years; range, 36–86 years). Of the 99 metastatic breast samples, 70 (70.7%) were HR-positive/HER2 Low, 21 (21.2%) were HR-positive/HER2 Ultralow, and 8 (8.1%) were HR-positive/HER2 Null. The most common sites of metastasis sampled included bone (57 of 99 cases; 58%), liver (19 of 99 cases; 19%), lung (7 of 99 cases; 7%), and soft tissue (5 of 99 cases; 5%). Primary tumor HER2 status was significantly associated with metastatic HER2 status ($P = 0.03$); there were no other significant feature differentiating HR-positive/HER2 Low, HR-positive/HER2 Ultralow, and HR-positive/HER2 Null. Cytotoxic chemotherapy was administered to 31 (55.4%) of 56 patients with biomarker results on both primary and metastatic tumor samples; there was no significant difference between HER2 Low, Ultralow, and Null status in primary and metastatic tumor samples.

3.2. Concordance of HER2 status between primary breast carcinoma and metastatic breast carcinoma samples

Primary tumor hormone receptor status and HER2 status were available in 56 (56.6%) of 99 cases (Fig. 1). Primary tumor biomarker testing was performed on core biopsy in 55 (98.2%) cases and excision in 1 (1.8%) case. Biomarker status between primary breast tumor and metastatic breast carcinoma samples was concordant in 39 (70%) of 56 cases, with a majority of HER2 Low primary tumors showing HER2 Low status in the metastatic setting (37 of 49 HER2 Low cases; 75.5%). Most common alteration in HER2 status amongst the hormone receptor-positive cases included HER2 Low to HER2 Ultralow (10 of 49 cases; 20.4%), HER2 Null to HER2 Ultralow (2 of 4 cases; 50%), and HER2 Ultralow to HER2 Null and to HER2 Low (1 of 3 cases each; 33.3% each), though the latter two constitutes make an overall small number of cases.

3.3. Patients with hormone receptor-positive, HER2 Low metastatic breast carcinoma

Of the 70 HR-positive/HER2 Low metastatic tumor cases, median patient age was 70 years (mean, 67 years; range, 37–83 years), and median primary tumor size was 3.0 cm (mean, 3.7 cm; range, 0.1–12.0 cm). Primary tumor histology was invasive carcinoma of no special type in 39 (56%) of 70 cases, invasive lobular carcinoma and its variants in 14 (20%) cases, and invasive carcinoma with mixed mammary ductal and lobular features in 10 (14%) cases; primary tumor histology type was not available in 7 of 70 (10%) cases. Primary tumor Nottingham grade was most commonly 2 (31 of 70 cases; 44.3%), followed by 3 (21 of 70 cases; 30.0%) and I (8 of 70 cases; 11.4%); primary tumor Nottingham grade was not available in 10 (14.3%) of 70 cases. Axillary nodes at time of primary diagnosis were involved by metastatic carcinoma in 37 (52.9%) of 70 cases and uninvolved 19 (27.1%) of 70; axillary nodal status was

Table 1
Comparison of clinical and pathologic features of 99 cases from 95 patients with metastatic hormone receptor-positive breast carcinoma by HER2 Low, Ultralow, and Null status.

	HR+, HER2 Low ($n = 70$)	HR+, HER2 Ultralow ($n = 21$)	HR+, HER2 Null ($n = 8$)	Total	*P*
Age, median (mean) [range], y	70 (67) [37–83]	68 (68) [46–86]	62 (60) [36–82]	69 (67) [36–86]	0.26
Primary tumor size, median (mean) [range], cm	3.0 (3.7) [0.1–12.0]	2.2 (2.5) [0.4–9.0]	2.4 (2.3) [0.2–4.3]	2.7 (3.3) [0.1–12]	0.14
Primary tumor histology, *n*/total (%)					0.39
Invasive ductal carcinoma and variants	39/70 (56)	13/21 (61.9)	3/8 (37.5)	55/99 (56)	
Invasive lobular carcinoma and variants	14/70 (20)	3/21 (14.3)	3/8 (37.5)	20/99 (20)	
Invasive mammary carcinoma with mixed ductal and lobular features	10/70 (14)	1/21 (4.8)	2/8 (25)	13/99 (13)	
NA	7/70 (10)	4/21 (19)	0/8 (0)	11/99 (11)	
Primary tumor histologic grade, *n* (%)					0.56
1	8/70 (11.4)	3/21 (14.3)	3/8 (37.5)	14/99 (14.1)	
2	31/70 (44.3)	9/21 (42.9)	3/8 (37.5)	43/99 (43.5)	
3	21/70 (30.0)	5/21 (23.8)	2/8 (25.0)	28/99 (28.3)	
NA	10/70 (14.3)	4/21 (19.0)	0/8 (0)	14/99 (14.1)	
Primary tumor biomarker status, *n*/total (%)					0.03*
HR+, HER2 low	37/39 (95.0)	10/13 (76.9)	2/4 (50)	49/56 (87.5)	
HR+, HER2 Ultralow	1/39 (2.5)	1/13 (7.7)	1/4 (25)	3/56 (5.4)	
HR+, HER2 Null	1/39 (2.5)	2/13 (15.4)	1/4 (25)	4/56 (7.1)	
Lymph node involvement, *n* (%)					1.0
Yes	37/70 (52.9)	9/21 (42.9)	3/8 (37.5)	49/99 (49.5)	
No	19/70 (27.1)	5/21 (23.8)	2/8 (25.0)	26/99 (26.3)	
NA	14/70 (20.0)	7/21 (33.3)	3/8 (37.5)	24/99 (24.2)	
Cytotoxic chemotherapy, *n* (%)					0.35
Received	42/70 (60)	16/21 (76)	4/8 (50)	62/99 (63)	
Did not receive	27/70 (39)	5/21 (24)	4/8 (50)	36/99 (36)	
NA	1/70 (1)	0/21 (0)	0/8 (0)	1/99 (1)	
Endocrine therapy, *n* (%)					0.12
Received	59/70 (84.3)	21/21 (100)	8/8 (100)	88/99 (89)	
Did not receive	10/70 (14.3)	0/21 (0)	0/8 (0)	10/99 (10)	
NA	1/70 (1.4)	0/21 (0)	0/8 (0)	1/99 (1)	
Clinical status, *n* (%)					0.72
Alive with metastatic disease	47/70 (67)	12/21 (57)	5/8 (62.5)	64/99 (65)	
Progression of disease	5/70 (7)	3/21 (14)	1/8 (12.5)	9/99 (9)	
Died of disease	18/70 (26)	6/21 (29)	2/8 (25.0)	26/99 (26)	
Clinical follow-up, median (mean) [range], m	90 (110) [0–353]	89 (95) [3–317]	47 (89) [3–400]	82 (105) [0–400]	0.70

Abbreviations: m, months; NA, not applicable or available; y, years.
* Statistically significant *P* value < 0.05.

not known in 14 (20.0%) of 70 cases. Primary tumor biomarker status was available in 39 (56%) of 70 HR-positive/HER2 Low cases. Primary tumor biomarker status was HR-positive/HER2 Low in 37 (95%) of 39 cases, HR-positive/HER2 Ultralow in 1 (2.5%) case, and HR-positive/HER2 Null in 1 (2.5%) case. Median time to metastasis was 105 months (mean, 121 months; range, 2–385 months); 10 cases diagnosed as *de novo* metastasis. Median clinical follow-up time was 90 months, and 47 (67%) of 70 patients were alive with disease, 5 (7%) patients showed disease progression, and 18 (26%) patients died of disease.

3.4. Patients with hormone receptor-positive, HER2 Ultralow metastatic breast carcinoma

Of the 21 HR-positive/HER2 Ultralow metastatic tumor cases, median patient age was 68 years (mean, 68 years; range, 46–86 years), and median primary tumor size was 2.2 cm (mean, 2.5 cm; range, 0.4–9.0 cm). Primary tumor histology was invasive carcinoma of no special type in 13 (62%) of 21 cases, invasive lobular carcinoma and its variants in 3 (14%) cases, and invasive carcinoma with mixed mammary ductal and lobular features in 1 (5%) case; primary tumor histology type was not available in 4 of 21 (19%) cases. Primary tumor Nottingham grade was most commonly 2 (9 of 21 cases; 42.9%), followed by 3 (5 of 21 cases; 23.8%) and 1 (3 of 21 cases; 14.3%); primary tumor Nottingham grade was not available in 4 (19.0%) of 21 cases. Axillary nodes at time of primary diagnosis were involved by metastatic carcinoma in 9 (42.9%) of 21 cases and uninvolved in 5 (23.8%) of 21; axillary nodal status was not known in 7 (33.3%) of 21 cases. Primary tumor biomarker status was available in 13 (61.9%) of 21 HR-positive/HER2 Ultralow cases. Primary tumor biomarker status was HR-positive/HER2 Low in 10 (77%) of 13 cases, HR-positive/HER2 Null in 2 (15%) cases, and HR-positive/HER2 Ultralow in 1 (8%) case. 21-gene recurrence score assay was performed and available in 5 (24%) of 21 cases. Results of 21-gene recurrence score was intermediate risk in 4 (80%) cases and high risk in 1 (20%) case; no HR-positive/HER2 Ultralow case had a low risk 21-gene recurrence score. Median time to metastasis was 87 months (mean, 98 months; range, 3–306 months); 3 cases diagnosed as *de novo* metastasis. Median clinical follow-up time was 89 months, and 12 (57%) of 21 patients were alive with disease, 3 (14%) patients showed disease progression, and 6 (29%) patients died of disease.

3.5. Patients with hormone receptor-positive, HER2 Null metastatic breast carcinoma

Of the 8 HR-positive/HER2 Null metastatic tumor cases, median patient age was 62 years (mean, 60 years; range, 36–82 years), and median primary tumor size was 2.4 cm (mean, 2.3 cm; range, 0.2–4.3

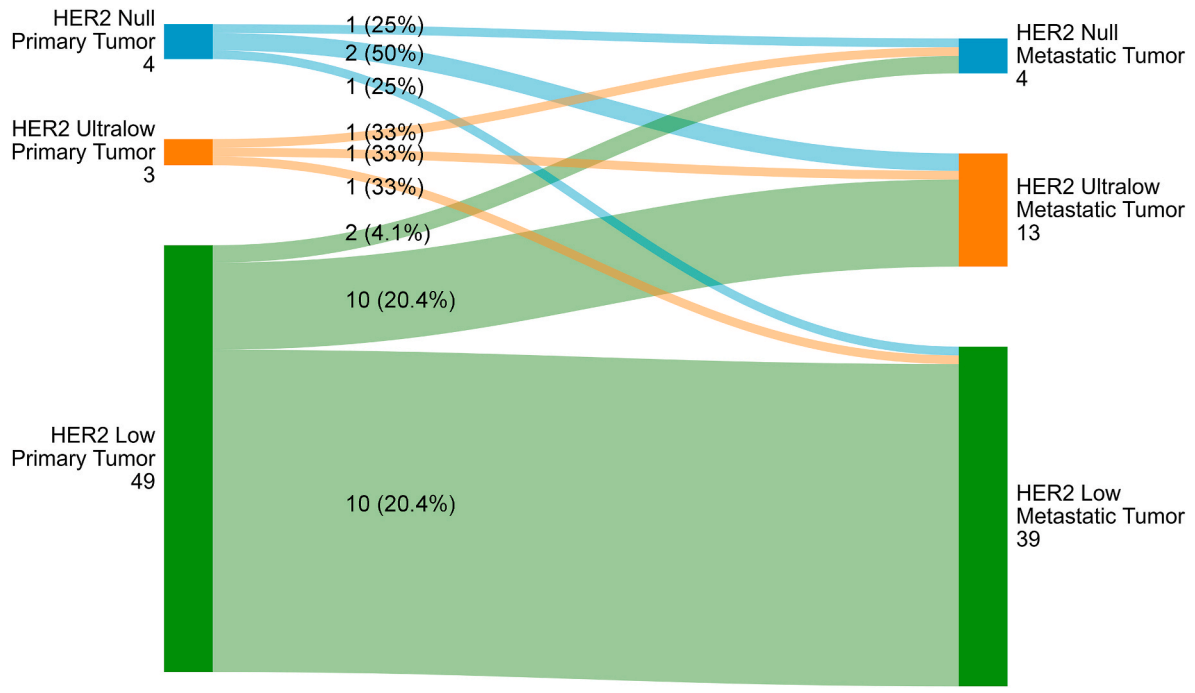

Fig. 1. Comparison of human epidermal growth factor receptor 2 (HER2) expression levels in 56 hormone receptor-positive primary and metastatic breast carcinoma samples.

cm). Primary tumor histology was invasive carcinoma of no special type in 3 (37.5%) of 8 cases, invasive lobular carcinoma and its variants in 3 (37.5%) cases, and invasive mammary carcinoma with mixed ductal and lobular features in 2 (25%) cases. Primary tumor Nottingham grade was most commonly 1 (3 of 8 cases; 37.5%) and 2 (3 of 8 cases; 37.5%), followed by 3 (2 of 8 cases; 25%). Axillary nodes at time of primary diagnosis were involved by metastatic carcinoma in 3 (37.5%) of 8 cases and uninvolved in 2 (25%) of 8; axillary nodal status was not known in 3 (37.5%) of 8 cases. Primary tumor biomarker status was available in 4 (50%) of 8 HR-positive/HER2 Null cases. Primary tumor biomarker status was HR-positive/HER2 Low in 2 (50%) of 4 cases, HR-positive/HER2 Ultralow in 1 (25%) case, and HR-positive/HER2 Null in 1 (25%) case. 21-gene recurrence score assay was performed and available in 3 (37.5%) of 8 cases. Results of 21-gene recurrence score assay was intermediate risk in 2 (66.7%) cases and high risk in 1 (33.3%) case; no HR-positive/HER2 Null case had a low risk 21-gene recurrence score. Median time to metastasis was 74 months (mean, 126 months; range, 35–383 months); 3 cases diagnosed as *de novo* metastasis. Median clinical follow-up time was 47 months, and 5 (62.5%) of 8 patients were alive with disease, 1 (12.5%) patient showed disease progression, and 2 (25%) patients died of disease.

4. Discussion

In this one-year retrospective, single-institution study of metastatic hormone receptor-positive breast carcinoma, we have demonstrated that approximately 60% of cases were HER2 Low and 20% were HER2 Ultralow, indicating a large proportion of patients who would benefit from HER2-directed antibody drug conjugates. We have shown that approximately one-third of cases show discordant HER2 status between primary and metastatic tumor samples. Although most of the discrepancies would still render the patient eligible for HER2-directed antibody drug conjugates in the new HER2 therapy landscape, a minority of patients would have a clinically meaningful change in HER2 status (e.g., HER2 Low to HER2 Null or HER2 Null to HER2 Ultralow). We have also found no significant difference in clinical and pathologic features or clinical outcome amongst the HER2 Low, HER2 Ultralow, and HER2 Null cases in the metastatic setting, other than primary tumor HER2 status ($P = 0.03$).

While the DESTINY-Breast04 and DESTINY-Breast06 trials focused on patients with HR-positive/HER2 Low and HR-positive/HER2 Ultralow metastatic breast carcinoma, recent studies characterizing the clinical and pathologic features of patients in the HER2 low landscape include tumors with and without HR expression, without differentiating between HER2 Low, HER2 Ultralow, and HER2 Null [3–5]. Herein, we specifically included patients with metastatic HR-positive breast carcinoma, demonstrating a large portion would be eligible for and could benefit from novel HER2-directed antibody drug conjugates.

Almstedt and colleagues reported HER2 expression discrepancy rate of 43% (64 of 148 cases) between primary and metastatic breast carcinoma samples [3]. Similarly, the Fudan group observed a discordance rate of 28% (27 of 98 cases) and found that HER2 discordance was significantly associated with T stage [4]. Yang et al. found a HER2 discordance rate of 37% (62 of 169 cases) [5]. In our cohort, HER2 discordance amongst HR + breast carcinoma was 30% (17 of 56 cases), taking into account the distinction between HER2 Low, HER2 Ultralow, and HER2 Null. Given that prior studies did not differentiate between these categories, some tumors previously classified as HER2 IHC 0 may now be reclassified as HER2 Ultralow, potentially making these patients eligible for ADC therapy. Consequently, previously reported discrepancy rates between primary and metastatic tumors may not fully capture the therapeutic implications of HER2 status shifts within the evolving treatment landscape.

Several factors may contribute to differences in HER2 status between primary and metastatic samples: intratumoral heterogeneity, size of tissue sample tested, biologic effects during disease progression or treatment, and variety of pre-analytical and analytic factors—particularly HER2 IHC assay performance [17–19]. These factors are especially crucial when assessing low levels of HER2 IHC expression, underscoring the need for standardized testing methodologies to ensure accurate classification and treatment selection.

Another key challenge in classifying HER2 Low, HER2 Ultralow, and HER2 Null breast carcinoma is the reproducibility of these designations, both within and across laboratories [20]. While this aspect of HER2 IHC

testing is outside the scope of this study, prior research have highlighted significant interobserver variability amongst pathologists scoring low-level HER2 expression, as well as inconsistencies in immunoreactivity when assays are repeated in the same laboratory or performed in different laboratories [21–25]. These variations may be influenced by factors such as differences in antigen retrieval, fixation times, staining protocols, and scoring thresholds. In this study, we have made efforts to control for many potential sources of variability, including central testing of tissue samples using the standardized validated protocols and joint assessment of cases with low-level HER2 IHC expression (i.e., score of 0). While alternative methods for detecting low-level HER2 expression are under development [26], HER2 IHC and ISH remain the only clinical validated tests for assessing HER2 Low, HER2 Ultralow, and HER2 Null status of breast carcinoma. Despite our quality control measures, we acknowledge inherent assay variability remains a limitation, and as such, our results may not fully reflect the true biological processes in the tested samples.

Identification of HER2 Low, HER2 Ultralow, and HER2 Null breast carcinoma holds significant potential for guiding treatment decisions, particularly in the context of antibody-drug conjugate (ADC) therapy. At present, the primary rationale for recognizing these categories is to identify patients who may benefit from such treatments. However, the clinical significance of these categories remains uncertain, particularly because many clinical trials have excluded HER2 Null cancers from participation. As a result, there is limited data on whether patients with these tumors would derive benefit from ADC therapy. Notably, the phase II Daisy trial (DB-06) is one of the few studies to include HER2 IHC 0 cases, which presumably included both HER2 Ultralow and Her2 Null tumors [27]. The results from this trial suggest that some patients with HER2 0 tumors show clinical benefit to ADC therapy, potentially due to ADC uptake by even minimal levels of HER2 expression and subsequent tumor cell death via the bystander effect [28,29].

It is important to clarify that the term ‘HER2 Null’ does not imply complete absence of HER2 expression in tumor cells, and absence of detectable HER2 expression on IHC does not necessarily equate to a total lack of the protein. Normal breast epithelial cells express HER2, and a lack of immunoreactivity in standard HER2 testing does not necessarily indicate that tumor cells are entirely devoid of HER2 protein expression [26]. To that end, some have advocated for the term ‘HER2 Normal’ rather than ‘HER2 Negative’ to more accurately reflect the baseline expression of HER2 in normal cells. Consequently, the necessity of distinguishing between subcategories of HER2 negativity for treatment eligibility may become less relevant, particularly as patients with breast carcinoma of low level HER2 expression have demonstrated clinical benefit from ADC therapy. However, further clinical trials are required to determine the true significance of these HER2 subcategories in guiding treatment decisions.

Patients with unresectable or metastatic breast carcinoma with HER2 Low and Ultralow are currently qualified for HER2 antibody drug conjugate therapy, such as trastuzumab deruxtecan; however, it remains uncertain whether HER2 Low, Ultralow, and Null IHC interpretations truly represent biological differences or merely reflect the lower limits of the IHC assay’s analytical sensitivity (*vide supra*). To that end, despite recent developments in the HER2 treatment paradigm with the advent of such HER2-directed antibody drug conjugates, current ASCO/CAP HER2 testing guideline still maintain the traditional three categories of immunohistochemical scoring system of “Negative” for IHC scores of 0 and 1+, “Equivocal” for 2+, and “Positive” for 3+. A recent update from ASCO/CAP states that there is no evidence for HER2 Low as a prognostic and predictively distinct category—similar to the findings we have shown herein. The guideline panel does acknowledges indications for HER2-targeted therapy in the setting of HER2 Low breast carcinoma and concedes the clinically relevance of distinguishing HER2 IHC 0 and 1+ [30]. The European Society for Medical Oncology (ESMO) suggests that patients with tumor biopsies showing HER2 IHC score 0 should undergo repeat HER2 testing on additional tumor samples, as this may “open new therapeutic opportunities.” [31].

The major limitation of this study is lack of complete immunohistochemistry for primary tumor breast biomarkers in nearly half of study cohort cases. This was primarily due to either patient’s primary tumor diagnosed during the period when our institution’s laboratory performed FISH as the primary HER2 testing methodology or patient’s primary tumor breast biomarkers were performed at an outside institution. Furthermore, this study was limited by the number of patients with HR-positive metastatic breast carcinoma, though comparatively, our study demonstrated similar rates of HER2 discordance as other recent studies.

Despite these limitations, our small, retrospective single-institution study provides a unique insight into the HER2 landscape in the HR-positive metastatic breast carcinoma environment and highlights the importance of biomarker testing on metastatic samples. Herein, we demonstrated similar clinical outcomes in patients with HR+/HER2 Low, HR+/HER2 Ultralow, and HR+/HER2 Null metastatic breast carcinoma. Further investigation is warranted to identify if treatment with HER2-directed antibody drug conjugates contributes to substantial differences in outcome.

CRediT authorship contribution statement

Raza S. Hoda: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Patrick J. McIntire:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation.

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Conflict of interest statement

The authors have no relevant conflicts of interest to disclose.

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