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Review Article

From Negative to Low: Understanding HER2-Low Breast Cancer

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Abstract

Breast cancer (BC) is the most common solid organ malignancy among women globally, with HER2 playing a notable role in its molecular classification and treatment. Traditionally, only HER2-positive tumors (IHC 3+ or IHC 2+/ISH+) were eligible for targeted therapy. However, emerging research has identified a subset of BC that have been defined as HER2-low (IHC 1+ or 2+/ISH−). These BC cases historically have been categorized as HER2-negative and not considered eligible for targeted therapy. However, this subset has shown potential responsiveness to newer antibody-drug conjugates (ADCs) like trastuzumab deruxtecan (T-DXd). This study aims to review the evolving concept of HER2-low BC in terms of its definition, diagnostic challenges, molecular features, prognostic significance, and recent therapeutic advancements, particularly the ADC-based targeted therapy. A comprehensive literature review was conducted for publications from 2015 to 2025 using peer-reviewed journals and authoritative guidelines such as ASCO, CAP, and NCCN. Studies addressing HER2-low diagnostic criteria, molecular characterization, clinical trials, treatment outcomes, and diagnostic challenges were included. Data extraction focused on HER2-low definitions, prevalence, PAM50/MammaPrint-based molecular profiling, ADC efficacy, interobserver variability, and implications of HER2 IHC heterogeneity. HER2-low BC accounts for approximately 40 to 50% of all BCs and is more prevalent in hormone receptor-positive tumors. Molecular profiling reveals that HER2-low cancers are mainly luminal A/B subtypes, with some basal-like and HER2-enriched tumors. The clinical benefit of T-DXd in HER2-low metastatic BC was confirmed by the DESTINY-Breast04 trial, significantly improving progression-free and overall survival. However, accurate HER2-low identification remains challenging due to intratumoral heterogeneity, borderline IHC scoring (particularly between 0 and 1+), and poor interobserver concordance (as low as 26%). Diagnostic pitfalls arise from staining variability, technical artifacts, and differences between primary and metastatic sites. Emerging digital image analysis and machine learning tools show promise in refining HER2 IHC interpretation but require further validation. HER2-low BC has emerged as a distinct and clinically actionable entity due to

Keywords

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the efficacy of HER2-targeted ADCs. This shift necessitates precise HER2 assessment by pathologists, with emphasis on distinguishing IHC 0 from 1+ and standardizing preanalytical and analytical protocols. Enhanced diagnostic accuracy is essential to guide appropriate therapy and support the expanding landscape of personalized BC treatment. Future updates in testing guidelines and integration of digital tools may further optimize HER2-low classification and patient outcomes.

Introduction

The most common solid organ cancer and the second leading cause of cancer-associated mortality for women worldwide is breast cancer (BC).[1] In India, BC accounts for 28.2% of all cancers among females.[2] Precision medicine has transformed the management of BCs, and molecular subtyping plays a major role in treatment selection and prognosis. In the past 30 years, the ERBB2 gene, HER2, has changed from being a predictor of poor prognosis to a factor in patient stratification for HER2-targeted treatments, particularly trastuzumab, when HER2 is overexpressed or amplified in tumor cells.[3]

A positive HER2 expression is defined as immunohistochemistry score 3+ (IHC 3+) or IHC 2+/in situ hybridization showing positive (ISH+), while a negative HER2 is described as IHC scoring 0, IHC scoring 1+, or IHC scoring 2+/ISH negative, according to the scoring guidelines used in accordance with the 2018 guidelines of American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).[4,5]

Approximately 10 to 20% of BC show HER2 positivity, whereas 80 to 90% are classified as HER2-negative.[6] Traditionally, only tumors that overexpress HER2 (IHC 3+) or show amplification (ISH +) were considered eligible for targeted therapies (►**Fig. 1A, B**). HER2-negative tumors were defined as those that lacked these characteristics. However, it has been noted that a sizable fraction of HER2-negative tumors, now known as HER2-low, do show decreased HER2 expression.[7] The current sanction of the antibody-drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) for BC with metastases with HER2-low expression has raised awareness of this subgroup.[8,9] According to recent clinical trials, T-DXd-based ADC is beneficial for BC with decreased HER2 protein expression.[10] T-DXd-based ADC was shown to be therapeutically beneficial in patients with HER2-low resistant metastatic BC in the DESTINY-Breast04 Phase III clinical study. The study found that, in comparison to current therapies, the risk of illness progression was decreased by 50% and the BC-specific mortality was decreased by 36%.[9] These results have redefined therapeutics for this subset of cases (►**Table 1**). Parallelly, the accurate identification and reporting of HER2-low status by IHC have become an important responsibility for pathologists, bringing substantial changes in HER2 testing protocols and interpretation standards.[11]

Materials and Methods

To track the development of HER2 testing guidelines and the identification of BC with HER2-low expression as a discrete clinical subtype, the literature search encompassed papers

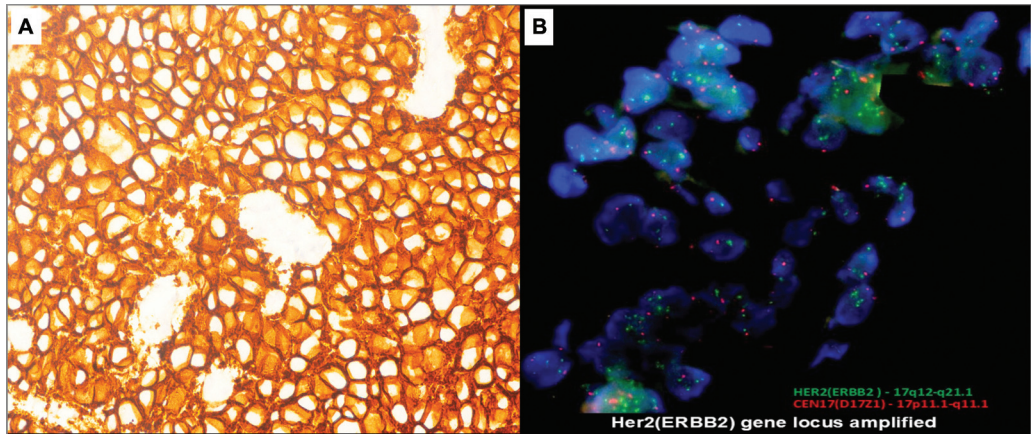

Fig. 1 Photomicrograph shows (**A**) HER2 IHC stain (200× magnification): strong, complete membranous expression for HER2 protein in more than 10% cells. (**B**) The FISH image shows HER2 gene amplification. ERBB2/CEN 17 dual color amplification probe showing multiple copies of green signals (HER2/ERBB2 gene locus, 17q12-q21.1), indicating high-level amplification, where red signals represent the chromosome 17 α satellite centromeric region. The HER2/CEP17 ratio is > 2.0, diagnostic of HER2 amplification according to ASCO/CAP guidelines.

Table 1 HER2 status and therapeutic implications

HER2 status	IHC/ISH interpretation	Therapeutic implications
HER2 0	No staining or incomplete faint membrane staining in $\leq$ 10% cells	Considered HER2-negative; not eligible for HER2-targeted therapy
HER2 1+	Incomplete faint/barely perceptible membrane staining in $>$ 10% cells	HER2-low; potential eligibility for novel ADCs
HER2 2+/ISH−	Weak to moderate complete membrane staining in $>$ 10% cells; ISH not amplified	HER2-low; potential eligibility for novel ADCs, but not for trastuzumab
HER2 2+/ISH+	Weak to moderate complete membrane staining in $>$ 10% cells; ISH amplified	HER2-positive; eligible for standard HER2-targeted therapies like trastuzumab
HER2 3+	Strong complete membrane staining in $>$ 10% cells	HER2-positive; eligible for standard HER2-targeted therapies like trastuzumab

from 2003 to 2025. Preference was given to studies published in reputable, high-impact medical journals as well as documents issued by leading guideline organizations such as ASCO, CAP, and NCCN. Peer-reviewed clinical research formed the core of the evidence base. Clinical trials were reviewed and organized according to their phase and their specific applicability to the HER2-low subgroup.

Inclusion Criteria

1. Original research articles, review articles, clinical trials, consensus guidelines, and regulatory agency documents discussing HER2-low BC.
2. Studies addressing diagnostic criteria, IHC scoring, gene amplification, molecular characteristics, treatment outcomes, and prognostic relevance.
3. Both early-stage and metastatic HER2-low BC were considered.

Exclusion Criteria

Studies focusing solely on HER2-positive (IHC 3+/ISH-amplified) or HER2-negative (IHC 0) tumors without subgroup analysis for HER2-low. Data from the included studies were extracted for the following purposes:

1. Definition and diagnostic criteria of HER2-low BC: historical and current definitions, with reference to ASCO and the CAP guidelines.
2. Prevalence and clinicopathological features: worldwide and local prevalence data, including data from the Indian subcontinent.
3. Molecular profiling: PAM50 classification, MammaPrint data, and genomic landscape
4. Therapeutic implications: drug efficacy and therapeutic safety of ADC, like T-DXd
5. Treatment responsiveness and prognostic significance: outcomes in HER2-low compared with HER2-zero BC
6. Challenges in HER2-low classification: interobserver variability, IHC scoring issues, and heterogeneity.

A total of 73 references were selected after careful review, and emphasis was placed on the most recent high-impact studies, clinical trials, and guideline updates to ensure the review reflects current understanding and practice.

HER2-Low Breast Cancer: Evolving Concepts

Approximately 40 to 50% of BC exhibit some level of HER2 protein, IHC 1+ or greater.[12,13] The U.S. Food and Drug Administration (FDA) has given approval to trastuzumab and other HER2-specific treatments for HER2-positive BC patients, who are defined as those showing HER2, IHC scoring 3+ or IHC scoring 2+ with ISH confirming targeted gene amplification.[4] HER2 low-expressing BC with no evidence of amplification of the gene under current guidelines are classified as HER2-negative and hence are not considered suitable for HER2-specific therapies. Within them, a significant subset also do not express hormone receptors (HR-negative) and are associated with adverse prognosis. In the past decade, there has been sustained interest in determining whether patients with low HER2 expression scoring could derive an advantage from therapies targeted toward HER2. The NSABP B-47/NRG Oncology Phase III trial examined the effects of adjuvant chemotherapy, either with or without trastuzumab, in 3,270 patients with low HER2 expression and high-risk primary BC. Combining trastuzumab with chemotherapy did not enhance outcomes for women with ISH-negative BC who had HER2 IHC scores of 1+ or 2+ over a median follow-up period of 46 months. Furthermore, studies could not detect a discernible rise in disease-free survival in invasive BC.[14] The first HER2-targeting ADC for advanced HER2-positive BC was ado-trastuzumab emtansine (T-DM1; KADCYLA, Genentech), which was permitted by the FDA in 2013.[15] Trastuzumab duocarmazine (SYD-985) and T-DXd were approved in 2018 and 2019, respectively. In patients with advanced or metastatic cancer who have HER2-low, these novel treatments have demonstrated notable antitumor efficacy and a manageable safety margin.[16,17]

Characterization of the HER2-Low Phenotype in BC

No universally accepted definition for HER2-low BC exists at present. Based on the importance of HER2 receptors on the tumor cell membranes, early research indicated that HER2-low BC were defined as those with HER2 IHC scoring 1+ or

IHC scoring 2+ and a negative ISH result for novel treatments targeting HER2.[10,15] Evidence suggests that HER2 IHC score 0, IHC 1+, IHC 2+, and IHC 3+ represent nearly 20,000, 100,000, 500,000, and 2,300,000 receptors per tumor cell, respectively.[18] Emerging interest in the HER2-low expression is clinically relevant with the advent of newly approved ADCs.[19] A recent study examined how HER2 expression levels affect the exposure of the ADC trastuzumab-valine-citrulline-monomethyl in cancer cells. The study found a strong relationship between HER2 receptor count and ADC uptake, starting with cell lines that had over 50,000 receptors per cell.[20] HER2-low BC is a relatively recent concept, and more is needed to completely understand and characterize it. Better, more precise methods are required to measure HER2 expression levels in these cancers, to ensure treatment effectiveness, and to recognize the right patients who could profit from newer therapies. A proposal for transitioning from the existing binary HER2 classification system in BC to a three-tier classification has been recommended, incorporating HER2-positive, HER2-low, and HER2-negative categories[11,21] (►**Fig. 2**).

Molecular Characterization of HER2-Low BC

BC is stratified into luminal A, luminal B, HER2-enriched, and basal-like molecular subtypes, which offer important understandings into tumor biology and play a significant role in guiding therapeutic strategies. The subtypes have been integrated into diagnostic evaluations, which are now regularly used in clinical practice.[22,23]

Molecular characteristics of HER2-low BC were studied by Schettini et al[22] and Agostinetto et al[3] using the PAM50 assay, a qRT-pathological complete response (pCR)-based test that profiles 50 genes. Their studies revealed that HER2-low BC are a diverse cluster, predominantly consisting of luminal A (50.8–56.9%) and luminal B (22.8–28.8%) subtypes, while HER2-enriched (3.5–3.6%) and basal-like subtypes (13.3–17.7%) were observed less frequently. Zhang et al[24] explored the genomic configuration of HER2-low BC utilizing the 70-gene MammaPrint and 80-gene Blueprint molecular microarray test. Their findings showed comparable patterns of molecular subtypes with previous studies. Despite using different tests, all studies found that luminal subtypes were most common in HR+/HER2-low tumors, while HER2-enriched and basal-like subtypes were not as prevalent. When HR-negative/HER2-low BC were analyzed independently, they predominantly exhibited basal-like characteristics, with a marginally increased prevalence of HER2-enriched tumors than those lacking hormone receptors and HER2 expression.[3]

In a separate study, Zhang et al conducted an analysis of genomic data from 523 BC cases using next-generation sequencing with a targeted 520-gene panel. Their findings indicated that HER2-low tumors showed a greater frequency of mutations within the PI3K-AKT signaling pathway in comparison to both HER2-positive and HER2-negative subtypes. The mutations in the PI3K–AKT pathway in HER2-low tumors have important therapeutic implications. Aberrant activation of this pathway is known to promote tumor growth, survival, and resistance to therapy, suggesting a potential role for PI3K–AKT inhibitors in this subset. The combinations of these pathway inhibitors with HER2-targeted ADCs are being explored to overcome resistance mechanisms, thereby offering a promising avenue for enhancing treatment efficacy in HER2-low disease. However, mutations in checkpoint regulators, the Fanconi anemia pathway, p53, and genes involved in cell cycle control were less frequently identified in HER2-low tumors than in HER2-

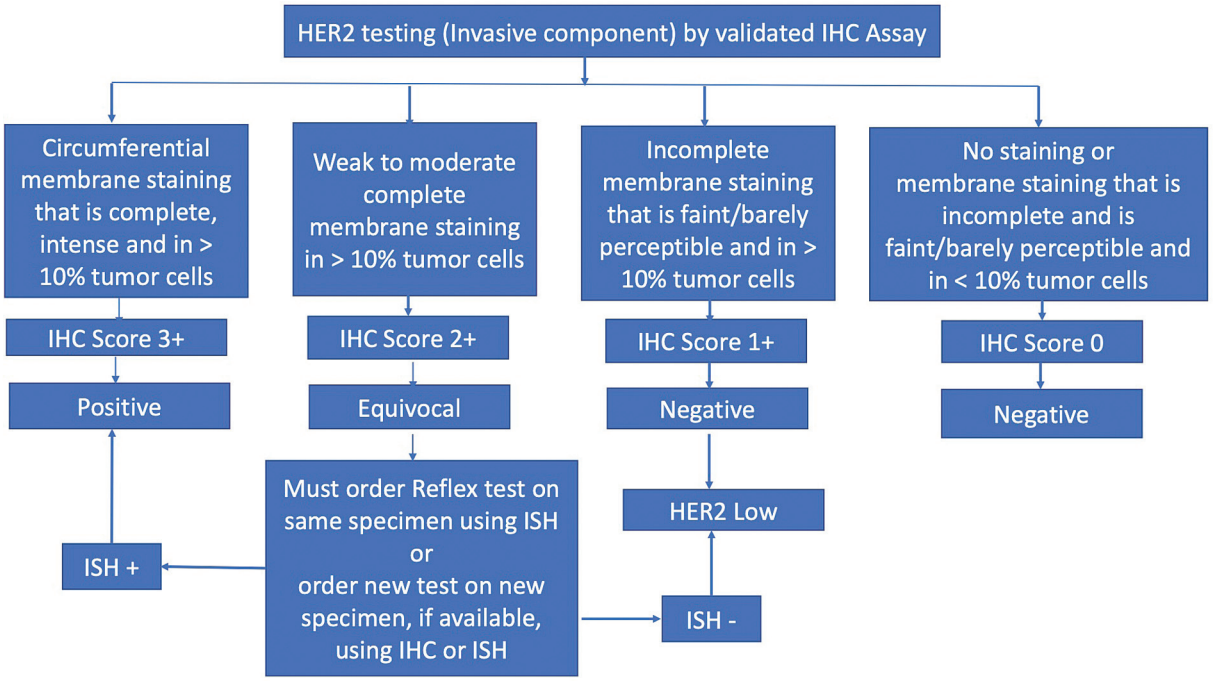

Fig. 2 The proposed HER2 "three-tier scoring system" in breast cancers (BC) to include HER2-low BCs.

negative counterparts. When HR-positive and HR-negative tumors were analyzed independently, no notable variation was found between HER2-low and HER2-negative tumors.[25] Currently, no studies have been published that evaluate whether HER2 serves as an active oncogene in HER2-low BC. The role of HER2 signaling activation in the pathophysiology of disease and progression of these tumors remains uncertain and continues to be a subject to explore.

Clinical Implications of HER2-Low BC

In comparison to HR-negative tumors, HER2-low BC is more frequently linked to HR positive tumors, and its frequency rises as HR expression does.[11,26] In contrast to HER2-negative tumors, HER2-low cancers generally show more pathological features in both HR-positive and HR-negative subtypes.[26–28] In HR-negative cancers, HER2-low tumors are infrequently basal-like,[3] more likely low-grade (35% vs. 18%), and express apocrine markers more frequently (57% vs. 36%).[29] Retrospective studies show mixed outcomes on prognosis. In early-stage, HR positive disease, some large studies report improved disease-free and overall survival among HER2-low compared with HER2-negative tumors,[27,30] while others found no difference.[11,31] HER2-low status appears more favorable in high-risk cases, such as those with high 21-gene recurrence scores[32] or those who did not receive adjuvant therapy. In HR negative tumors, most studies show no survival advantage,[3,11,28,30] though some[28,30] report better BC-specific or overall survival. Interestingly, HER2 +2/ISH-tumors in HR-negative cases may do worse than HER2 +1 or HER2-negative.[29] Evidence suggests that with neoadjuvant chemotherapy, HER2-low tumors in HR-positive cases show decreased pCR,[33] but HR-negative/HER2-low patients may have better survival, especially if they do not achieve pCR.[33,34] However, no survival difference has been observed in several studies.[35–38] HER2-low is more prevalent in HR-positive tumors in metastatic BC, and a large study found that these tumors had a somewhat better prognosis than HER2-negative ones.[39] In conclusion, HER2-low may be a good prognostic factor in some subgroups but does not always indicate better results in all contexts, indicating that it more accurately reflects tumor biology than causes it.

Clinical Developments: HER2-Based Targeted ADC Treatment for HER2-Low Breast Cancer

Standard HER2-targeted therapies require adequate HER2 receptor expression and activation of its downstream pathways. In HER2-low BC, such therapies, particularly trastuzumab, have shown limited benefit. This was shown in the Phase III NSABP-B47 trial, where clinical results in this subgroup were not improved by adding trastuzumab to adjuvant treatment.[14] ADCs were presented as a unique treatment approach to overcome the constraints caused by low and variable HER2 expression. A monoclonal antibody attached to a cytotoxic agent is present in ADCs. Chemotherapy can be delivered precisely because of its ability to connect to particular surface receptors that are expressed on tumor cells. After binding, the ADC is internalized, releasing the cytotoxic chemical inside the tumor cell while causing negligible harm to the nearby healthy tissue.[40] In the case of HER2-low tumors, the HER2 receptor serves primarily as a delivery mechanism for the drug, rather than the central target of antitumor activity. Nonetheless, a minimal threshold of HER2 expression is still necessary to facilitate this targeted delivery.

Trastuzumab emtansine (T-DM1), a second-generation ADC, was approved to treat HER2-positive metastatic BC and provided a basis for the advancement of HER2-targeted treatments. T-DM1 performed better in HER2-positive tumors than in HER2-low patients in a Phase II trial. T-DM1 offered preliminary evidence that HER2-targeted ADCs might be involved in conditions other than HER2-overexpressing malignancies, even though this was not explicitly examined in a prospective trial for HER2-low disease.[10] In patients with HER2-positive metastatic BC treated in the second-line scenario, T-DXd, a more effective HER2-directed ADC, has since shown improved efficacy over T-DM1. After the DESTINY-Breast04 trial's results, T-DXd became the first authorized ADC for the treatment of BC with low HER2 expression.[41] Through a cleavable tetrapeptide linker, trastuzumab is connected to deruxtecan, a topoisomerase I inhibitor, in this medication, which was originally known as DS-8201.[42] T-DXd has more potent anticancer activity than T-DM1 because of several characteristics, including a greater drug-to-antibody ratio, a cleavable linker, and a membrane-permeable payload that permits "bystander killing"[42,43] (►**Fig. 3**). This effect enables the cytotoxic drug to diffuse into and kill tumor cells with low or no HER2 expression, enhancing efficacy in tumors with heterogeneous HER2 distribution. T-DXd has shown therapeutic efficacy in treating HER2-low BC according to the DESTINY-Breast04 Phase III study.[9] Being the first anti-HER2 medication to work on tumors with low HER2 expression, it has led to the realization that ADC-based treatment may be sufficient even in cases where HER2 expression is modest. The findings also reinforce the relevance of the bystander effect in improving outcomes. However, whether T-DXd's antibody component contributes to HER2 signaling blockade in HER2-low tumors or simply acts as a delivery system remains unclear. Multiple ongoing clinical trials are exploring its role further in HER2-low disease.[44]

Pathologic Assessment of HER2 Breast Cancer: Diagnostic Pitfalls and Challenges for Pathologists

Under light microscopy, HER2 IHC is a semiquantitative technique that uses chromogenic labeling to identify membranous expression of the HER2 protein. According to current ASCO/CAP[45] guidelines, IHC is still the recommended first technique for HER2 testing. To ascertain HER2 status in cases where IHC is ambiguous (score 2+), further ISH confirmation is advised. These clinical practice guidelines do not

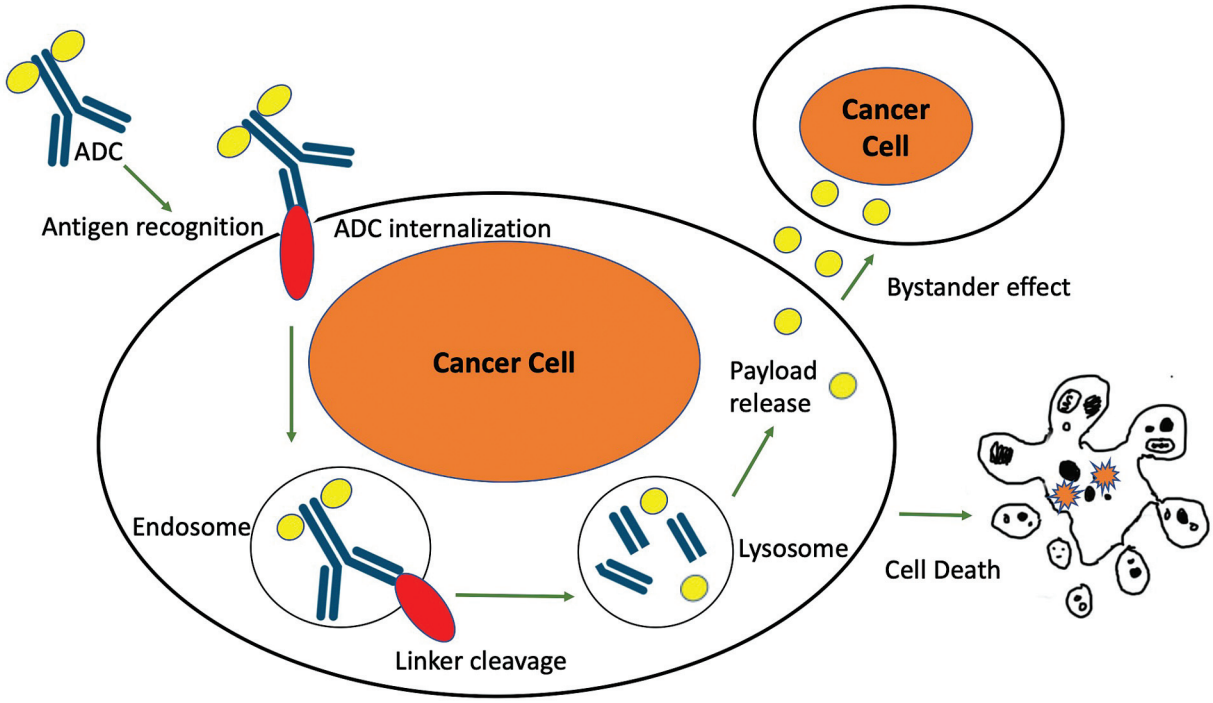

Fig. 3 Mechanism of ADC delivery and the bystander effect.

yet formally include the HER2-low group, though. With a 10% cutoff frequently used for classification, HER2 membranous expression in invasive breast carcinoma can vary significantly depending on the staining pattern (complete vs. incomplete), staining intensity (faint, weak, moderate, or strong), and the percentage of tumor cells exhibiting positivity. Additionally, tumors may display heterogeneous staining, with multiple intensities and patterns coexisting within the same specimen.

Multiple tumor cell subpopulations with different HER2 status coexisting within the same tumor are known as HER2 intratumoral heterogeneity.[46,47] Two different patterns of HER2 intratumoral heterogeneity—cluster or regional and mosaic or intermixed—have been identified. Separate populations of HER2-amplified and nonamplified tumor cells are characteristics of the clustered or regional type. Tumor cells that are both HER2-amplified and nonamplified are seen in the mosaic or intermixed form.[21,46,47] According to ISH and/or IHC, the prevalence of HER2 intratumoral heterogeneity in BC varies from study to study, ranging from 1 to 40%.[48,49] It is more prevalent in HER2-equivocal patients.[49,50] This variability of staining intensity across the spectrum further complicates the reproducibility and accuracy of HER2 scoring.[10] Regardless of their HER2 status, nontumorous cells around targeted HER2-expressing tumor cells experience the "bystander" death impact of the more recent HER2-targeting ADCs. The response to these new treatments may, therefore, be impacted by the types and existence of HER2 intratumoral heterogeneity.[15]

When more than 10% of tumor cells show uniform, strong, and full membranous staining, which is consistent with a score of 3+, HER2 IHC is usually easy to interpret (►**Fig. 1A**). However, evaluating tumors with heterogeneous staining intensities—ranging from weak to strong—can make assessment of the 10% threshold for strong positivity difficult. In such cases, the use of strong positive control tissues can assist pathologists in distinguishing true strong (3+) from moderate (2+) staining (►**Figs. 1A** and **4A**). When uncertainty persists regarding staining intensity, a conservative approach is advised, assigning a score of 2+ and recommending reflex testing with ISH to minimize the risk of false-positive HER2 classification.

Scoring equivocal (IHC 2+) cases remains more challenging than clearly positive (IHC 3+) or negative (IHC 0) interpretations, particularly for borderline cases near the 3+/2+ or 2+/1+ thresholds. The 2018 ASCO/CAP recommendations defined an IHC 2+ score as weak to moderate full membrane staining in more than 10% of invasive tumor cells to increase reproducibility[45] (►**Fig. 4A**). IHC score 2+ is frequently used to describe moderate to strong complete membranous staining seen in 10% or fewer tumor cells, even though this is not specifically stated in the formal criteria.[51] A small subpopulation of tumor cells with possible HER2 gene amplification may be present, according to this staining pattern. However, the limited proportion of these cells does not fulfill the criteria for a higher score. Additional tissue sampling in such cases could potentially change the proportion and impact of scoring outcomes.

Similarly, moderate to strong but incomplete membrane staining—such as lateral or basolateral patterns seen in over 10% of tumor cells, particularly in invasive micropapillary carcinomas, may also be scored as 2+. These cases require further evaluation by ISH testing.

Given that pathologists' concordance between IHC scores 0 and 1+ is occasionally as low as 26%, interobserver variability in evaluating HER2-low tumors continues to be a

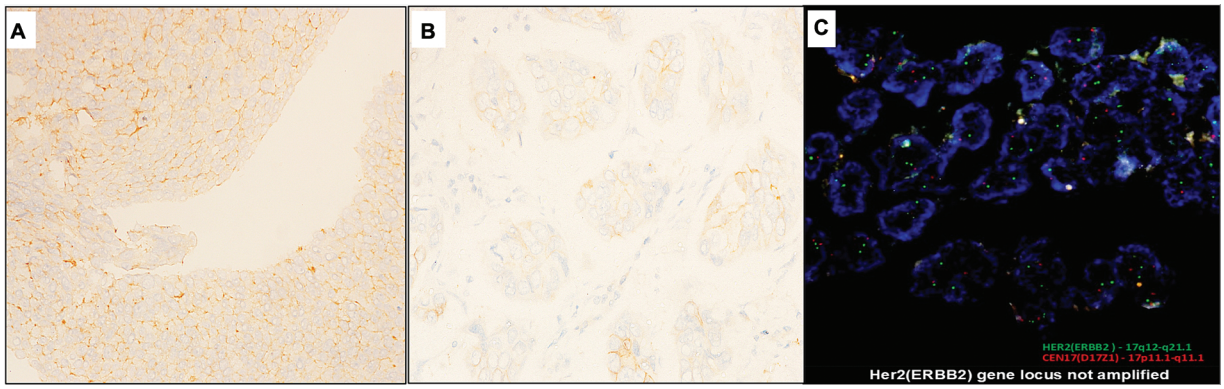

Fig. 4 Photomicrograph shows (**A**) HER2 IHC stain (200× magnification): moderate complete membranous expression for HER2 protein in more than 10% cells. (**B**) HER2 IHC stain (200× magnification): weak, incomplete membranous expression for HER2 protein in less than 10% cells. (**C**) FISH image showing no HER2 gene amplification. ERBB2/CEN 17 dual color amplification probe showing no amplification of green HER2/ERBB2 signals. The HER2/CEP17 ratio is approximately 1.0, indicating a nonamplified HER2 status.

major difficulty.[52,53] This variability often stems from difficulties in distinguishing between faint and weak staining intensities and accurately estimating their proportions. Additionally, the minimum threshold of HER2 protein expression required to elicit a response to ADC is still undefined.

Rare and atypical findings in BC include nuclear and cytoplasmic HER2 expression, with limited reports in the literature.[54,55] Nuclear HER2 localization, less commonly detected by IHC than immunofluorescence, may occur independently of membrane expression. In contrast to conventional membrane-bound signaling, there is evidence that HER2 can go to the nucleus and work in a transcriptional complex with STAT3, increasing cyclin D1 production and cell proliferation.[56] This nuclear presence has been linked to paclitaxel resistance,[57] and independently associated with poorer survival outcomes, potentially defining a biologically unique subset within HER2-positive tumors.[58] Cytoplasmic HER2 staining, as observed by Horiguchi et al in 34 of 1053 BC cases, was not linked to HER2 gene amplification on FISH but co-expressed neuroendocrine markers, suggesting a distinct phenotype.[54] Similar findings were documented by Safaei et al.[55] Some early studies attributed cytoplasmic localization to a technical artifact, particularly with the CB11 antibody clone.[59]

Since ADCs work by binding to HER2 on the surface receptors of tumor cells, there is a possibility that current IHC methods may not accurately detect low levels of HER2 expression. Defining BC as "HER2-low" remains challenging.[60] According to IHC scoring guidelines, a score of 1+ means there is faint, barely visible membrane staining in more than 10% of tumor cells (►**Fig. 2B**). Conversely, less than 10% of the cells in HER2-negative tumors exhibit either no staining at all or very weak staining[61] (►**Fig. 4B**). It is, therefore, understandably challenging to differentiate between these ratings. More than one-fifth of patients receiving neoadjuvant chemotherapy without anti-HER2 therapy[62] experienced discordance in HER2 IHC scoring between the resected small biopsy and the larger surgical specimen, and over 35% of patients experienced HER2-low IHC results that differed amongst primary tumors and metastatic relapses.[11,62]

Furthermore, the preanalytical, analytical, and postanalytical techniques that are now accessible have several drawbacks that could account for these inconsistent HER2 IHC results.[63,64] These include inter-observer variability,[65] true HER2 intratumoral expression heterogeneity,[49] true heterogeneity amongst various metastatic sites in HER2 expression level, and tissue sample processing that may impact protein detection rate,[66] all of which can lower the sensitivity and repeatability of HER2 testing.

The enzymatic activity of the detection system, tissue fixation, antigen retrieval procedures, antibody clones, ambient temperature, reaction time, and concentration of substrate are preanalytical parameters that significantly affect the HER2 staining intensity.[67] Accurate quantitative findings can't be obtained because of these IHC test limitations.[68,69]

The BC HER2 testing criteria were recently revised by the CAP and the ASCO. HER2 1+ thresholds are unchanged from the previous version of the guidelines, although tumors should be evaluated at 40× magnification with suitable controls, and slides should be reviewed by a second pathologist when scores are near zero. When making treatment decisions, medical oncologists are advised to take into account HER2-low results from any biopsy, not just from the current sample, because HER2 levels can vary.[9]

Approach to HER2-Low IHC Interpretation

According to the ASCO–CAP 2018 guidelines, testing for HER2 should be customized to detect BC that exhibits gene amplification or HER2 protein overexpression, as these are predictive indicators for HER2-targeted treatments.[62] Although the term "HER2-low" is not formally recognized in diagnostic terminology, pathologists are advised to include a comment in HER2 IHC and ISH reports. This should clarify that tumors classified as negative for HER2 (IHC scoring 0, 1+, or 2+/ISH showed no amplification (►**Fig. 4C**); lack overexpression or amplification but may still express low HER2 protein levels. Such cases, particularly IHC 1+ or 2+/ISH nonamplified, could qualify for

therapies that exploit HER2 for targeted drug delivery, like T-DXd (►**Fig. 2B, C, F**).

Despite IHC 0 and 1+ being grouped as HER2-negative under standard criteria, distinguishing them remains critical, as IHC 1+ tumors may be eligible for anti-HER2 ADCs. Accurate scoring should include the semiquantitative HER2 IHC result and follow best practices:

1. Apply the ASCO–CAP scoring system consistently.
2. Examine HER2 IHC-stained slides under high-power fields (40× objective) to better differentiate minimal (0) from faint (1+) membrane staining.
3. Seek advice from a second pathologist to increase diagnostic confidence in situations where interpretation falls between IHC scores 0 and 1+, particularly when over 10% of tumor cells exhibit weak, partial membrane staining.
4. Utilize external controls reflecting various expression levels, including 1+, to verify assay sensitivity.
5. Ensure optimal preanalytical handling of samples from both primary and metastatic tumors.

These measures help refine HER2 assessment in clinical settings while supporting therapeutic decision-making aligned with emerging treatment options[61]

Moreover, the era of digital image analysis can provide a solution to reduce interobserver variability in HER2 IHC interpretation. Studies have shown that artificial intelligence (AI tools) perform comparably to pathologists in identifying IHC 3+ cases and may slightly outperform them in distinguishing between IHC 0, 1+, and 2+ scores, though some 2+ cases were misclassified as 3+.[70,71] Machine learning could further refine HER2 quantification, especially in detecting subtle staining differences. In low-resource environments, HER2 scoring can be strengthened by incorporating digital pathology and AI through low-cost approaches such as smartphone-linked microscopes, camera attachments, and freely available software. Evidence from a recent multinational study showed that AI support enhanced diagnostic sensitivity and inter-observer consistency, with fewer errors compared with standard microscopy.[72] Although pathologists' judgment remains essential, it is observed that AI-based outputs improve reproducibility and can support standardized assessment. However, these technologies require thorough validation before clinical use, and current evidence is insufficient to recommend their routine implementation.

The strength of this study is its in-depth and up-to-date overview of HER2-low BC, integrating insights from recent advancements in clinical research, molecular diagnostics, and therapeutic interventions. Peer-reviewed sources and documents were included, ensuring both reliability and relevance. This study addresses the diagnostic challenges in HER2 assessment with practical solutions in the context of evolving diagnostic technologies. The limitations of this study are that it is a narrative review, so it does not include statistical synthesis or meta-analysis, which limits the ability to provide quantitative estimates or pooled outcomes. The scope for future investigation is the need for prospective studies to clarify whether HER2-low tumors play an oncogenic role or merely act as delivery sites for targeted agents. A deeper understanding of the molecular pathways in HR-negative, HER2-low tumors could facilitate the development of new therapeutic biomarkers.

Moreover, validation of digital image analysis systems and machine learning-based tools could help reduce scoring discrepancies in HER2 IHC.

The pathological and clinical behavior of HER2-low tumors, particularly their response to neoadjuvant therapy, is poorly understood, and this is one of the gray areas and research gaps. It is still unclear what level of HER2 expression is required for ADC to be effective. It is yet unclear how HER2-low classification might be used in early-stage disease management, including the design of adjuvant treatments, and this needs more research.

Conclusion

The recognition of HER2-low BC represents a notable advancement in both diagnostic categorization and treatment strategies of BC. This emerging category highlights the critical role of detailed pathological evaluation in supporting precision oncology. With the introduction of HER2-directed ADCs, precise detection of HER2-low expression has gained clinical relevance. Pathologists play a pivotal role in this process. Their primary duties include standardizing HER2 IHC protocols to reduce inter-laboratory variability, improving diagnostic precision by better differentiating between IHC scores of 0 and 1+, and making sure that HER2-low status is clearly documented in pathology reports with interpretive remarks. Moreover, there remains a need for better-defined and universally accepted HER2 expression thresholds to standardize the classification of HER2-low across laboratories and ensure reproducibility of results. This would accurately identify patients who may benefit from emerging HER2-targeted therapies. Nonetheless, effective communication and collaboration with oncology teams is essential to inform appropriate treatment strategies. It is also imperative that pathologists remain informed about updates in testing guidelines and emerging diagnostic technologies to provide the most effective patient care.

Authors' Contributions

Conceptualization: P.S.
Methodology: P.S., V.S., and N.G.
Writing—original draft preparation: P.S., V.S., and B.K.C.
Writing—review and editing: P.S., S.V., and T.T.
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Supervision: P.S. and V.S.

Ethical Approval

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki

Declaration and its later amendments or comparable ethical standards.

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Conflict of Interest
None declared.

References

1 Khalil MA, Habibian L, Martin C, et al. Landscape of HER2-low breast cancer: insights from a six-year study on prevalence and clinicopathological characteristics. Ann Diagn Pathol 2024; 72:152326
2 Sathishkumar K, Sankarapillai J, Mathew A, et al. Breast cancer survival in India across 11 geographic areas under the National Cancer Registry Programme. Cancer 2024;130(10):1816–1825
3 Agostinetto E, Rediti M, Fimereli D, et al. HER2-low breast cancer: molecular characteristics and prognosis. Cancers (Basel) 2021;13 (11):2824
4 Wolff AC, Hammond MEH, Allison KH, 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 2018;36 (20):2105–2122
5 Gradishar WJ, Moran MS, Abraham J, et al. Breast cancer, version 3.2024, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2024;22(05):331–357
6 Yao Y, Zhen H. Efficacy and prognosis of neoadjuvant chemotherapy in HER2 low-expressing breast cancer: a retrospective single-center study. Front Oncol 2024;14:1454726
7 Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer 2021;7(01):1
8 US Drug and Food administration. Approval Letter : Fam - Trastuzumab deruxtecan -nxki (BLA761139/5- 024). Silver Spring, MD: FDA; 2022
9 Modi S, Saura C, Yamashita T, et al. Trastuzumab deruxtecan in HER2-low metastatic breast cancer. N Engl J Med 2022;387(01): 9–20
10 Chia N, Gudi MA, Rakha E, Tan PH. HER2-low breast cancers: challenges in the interpretation of immunohistochemistry. Singapore Med J 2024. Doi: 10.4103/singaporemedj.SMJ-2023-037
11 Tarantino P, Gandini S, Nicolò E, et al. HER2-low breast cancer: pathologists' role in a new clinical setting. Mod Pathol 2022;35 (10):1468–1476
12 Rüschoff J, Friedrich M, Nagelmeier I, et al. Comparison of HercepTest mAb pharmDx (Dako Omnis, GE001) with Ventana PATHWAY anti-HER-2/neu (4B5) in breast cancer: correlation with HER2 amplification and HER2 low status. Virchows Arch 2022;481(05):685–694
13 Bardia A, Viale G. HER2-low breast cancer-diagnostic challenges and opportunities for insights from ongoing studies: a podcast. Target Oncol 2023;18(03):313–319
14 Fehrenbacher L, Cecchini RS, Geyer CE Jr, 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 2020;38(05):444–453
15 Zhang H, Katerji H, Turner BM, Hicks DG. HER2-low breast cancers. Am J Clin Pathol 2022;157(03):328–336
16 Banerji U, van Herpen CML, Saura C, 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 2019;20(08):1124–1135
17 Modi S, Park H, Murthy RK, 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 2020;38(17):1887–1896
18 Aidt F, Sierra M, Salomon K, Noumsi G. Comparing the sensitivity of HER2 epitope detection of HercepTest mAb pharmDx (Dako Omnis, GE001) and ventana pathway anti-HER-2/neu (4B5) using IHC calibrators. Appl Immunohistochem Mol Morphol 2024;32 (10):469–475
19 Curigliano G, Hu X, Dent RA, et al. Trastuzumab deruxtecan (T-DXd) vs physician's choice of chemotherapy (TPC) in patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-low or HER2-ultralow metastatic breast cancer (mBC) with prior endocrine therapy (ET): primary results from DESTINY-Breast06 (DB-06). J Clin Oncol 2024;42:1s–800s
20 Sharma S, Li Z, Bussing D, Shah DK. Evaluation of quantitative relationship between target expression and antibody-drug conjugate exposure inside cancer cells. Drug Metab Dispos 2020;48 (05):368–377
21 Marchiò C, Annaratone L, Marques A, Casorzo L, Berrino E, Sapino A. Evolving concepts in HER2 evaluation in breast cancer: heterogeneity, HER2-low carcinomas and beyond. Semin Cancer Biol 2021;72:123–135
22 Schettini F, Brasó-Maristany F, Kuderer NM, Prat A. A perspective on the development and lack of interchangeability of the breast cancer intrinsic subtypes. NPJ Breast Cancer 2022;8(01):85
23 Höller A, Nguyen-Sträuli BD, Frauchiger-Heuer H, Ring A. "Diagnostic and prognostic biomarkers of luminal breast cancer: where are we now?" Breast Cancer (Dove Med Press) 2023;15:525–540
24 Zhang H, Katerji H, Turner BM, Audeh W, Hicks DG. HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles. Mod Pathol 2022;35(08):1075–1082
25 Zhang G, Ren C, Li C, et al. Distinct clinical and somatic mutational features of breast tumors with high-, low-, or non-expressing human epidermal growth factor receptor 2 status. BMC Med 2022;20(01):142
26 Horisawa N, Adachi Y, Takatsuka D, et al. The frequency of low HER2 expression in breast cancer and a comparison of prognosis between patients with HER2-low and HER2-negative breast cancer by HR status. Breast Cancer 2022;29(02):234–241
27 Xu H, Han Y, Wu Y, et al. Clinicopathological characteristics and prognosis of HER2-low early-stage breast cancer: a single-institution experience. Front Oncol 2022;12:906011
28 Won HS, Ahn J, Kim Y, et al. Clinical significance of HER2-low expression in early breast cancer: a nationwide study from the Korean Breast Cancer Society. Breast Cancer Res 2022;24 (01):22
29 Jacot W, Maran-Gonzalez A, Massol O, et al. Prognostic value of HER2-low expression in non-metastatic triple-negative breast cancer and correlation with other biomarkers. Cancers (Basel) 2021;13(23):6059
30 Tan RSYC, Ong WS, Lee KH, et al. HER2 expression, copy number variation and survival outcomes in HER2-low non-metastatic breast cancer: an international multicentre cohort study and TCGA-METABRIC analysis. BMC Med 2022;20(01):105
31 Peiffer DS, Zhao F, Chen N, et al. Clinicopathologic characteristics and prognosis of ERBB2-low breast cancer among patients in the national cancer database. JAMA Oncol 2023;9(04):500–510
32 Almstedt K, Heimes AS, Kappenberg F, et al. Long-term prognostic significance of HER2-low and HER2-zero in node-negative breast cancer. Eur J Cancer 2022;173:10–19
33 Denkert C, Seither F, Schneeweiss A, 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 2021;22(08):1151–1161
34 Kang S, Lee SH, Lee HJ, et al. Pathological complete response, long-term outcomes, and recurrence patterns in HER2-low versus

HER2-zero breast cancer after neoadjuvant chemotherapy. Eur J Cancer 2022;176:30–40
35 de Moura Leite L, Cesca MG, Tavares MC, et al. HER2-low status and response to neoadjuvant chemotherapy in HER2 negative early breast cancer. Breast Cancer Res Treat 2021;190(01):155–163
36 Shao Y, Yu Y, Luo Z, et al. Clinical, pathological complete response, and prognosis characteristics of HER2-low breast cancer in the neoadjuvant chemotherapy setting: a retrospective analysis. Ann Surg Oncol 2022;29(13):8026–8034
37 Zhou S, Liu T, Kuang X, et al. Comparison of clinicopathological characteristics and response to neoadjuvant chemotherapy between HER2-low and HER2-zero breast cancer. Breast 2023;67:1–7
38 Domergue C, Martin E, Lemarié C, et al. Impact of HER2 status on pathological response after neoadjuvant chemotherapy in early triple-negative breast cancer. Cancers (Basel) 2022;14 (10):2509
39 de Calbiac O, Lusque A, Mailliez A, et al. Comparison of management and outcomes in ERBB2-low vs ERBB2-zero metastatic breast cancer in France. JAMA Netw Open 2022;5(09):e2231170
40 Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. Lancet 2019;394(10200):793–804
41 Cortés J, Kim SB, Chung WP, et al; DESTINY-Breast03 Trial Investigators. Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. N Engl J Med 2022;386(12):1143–1154
42 Ogitani Y, Aida T, Hagihara K, et al. DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. Clin Cancer Res 2016;22(20):5097–5108
43 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 2016;107(07):1039–1046
44 Shirman Y, Lubovsky S, Shai A. HER2-low breast cancer: current landscape and future prospects. Breast Cancer (Dove Med Press) 2023;15:605–616
45 Wolff AC, Hammond MEH, Allison KH, 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 2018;142(11):1364–1382
46 Hou Y, Nitta H, Li Z. HER2 intratumoral heterogeneity in breast cancer, an evolving concept. Cancers (Basel) 2023;15(10):2664
47 Muller KE, Marotti JD, Tafe LJ. Pathologic features and clinical implications of breast cancer with HER2 intratumoral genetic heterogeneity. Am J Clin Pathol 2019;152(01):7–16
48 Grassini D, Cascardi E, Sarotto I, et al. Unusual patterns of HER2 expression in breast cancer: insights and perspectives. Pathobiology 2022;89(05):278–296
49 Yamamoto Y, Yamada T, Ohkura N, et al. Equivocal (HER2 IHC 2+) breast carcinomas: gene-protein assay testing reveals association between genetic heterogeneity, individual cell amplification status and potential treatment benefits. Histopathology 2018;73 (05):767–775
50 Muller KE, Marotti JD, Tafe LJ. Pathologic features and clinical implications of breast cancer with HER2 intratumoral genetic heterogeneity: an institutional review. Am J Clin Pathol 2019;152 (01):7–16
51 Rakha EA, Tan PH, Quinn C, et al. UK recommendations for HER2 assessment in breast cancer: an update. J Clin Pathol 2023;76(04): 217–227
52 Fernandez AI, Liu M, Bellizzi A, et al. Examination of low ERBB2 protein expression in breast cancer tissue. JAMA Oncol 2022;8 (04):1–4
53 Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. NPJ Breast Cancer 2021;7(01):1
54 Horiguchi S, Hishima T, Hayashi Y, et al. HER-2/neu cytoplasmic staining is correlated with neuroendocrine differentiation in breast carcinoma. J Med Dent Sci 2010;57(02):155–163
55 Safaei A, Monabati A, Mokhtari M, Montazer M. Cytoplasmic Her2/neu immunohistochemical staining in breast cancer; from a molecular point of view. Iran J Pathol 2019;14(03):270–271
56 Béguelin W, Díaz Flaqué MC, Proietti CJ, et al. Progesterone receptor induces ErbB-2 nuclear translocation to promote breast cancer growth via a novel transcriptional effect: ErbB-2 function as a coactivator of Stat3. Mol Cell Biol 2010;30(23):5456–5472
57 Luo B, Wu XH, Feng YJ, et al. Nuclear HER2 contributes to paclitaxel resistance in breast cancer cells. Anticancer Drugs 2021;32(07):709–716
58 Schillaci R, Guzmán P, Cayrol F, et al. Clinical relevance of ErbB-2/HER2 nuclear expression in breast cancer. BMC Cancer 2012; 12:74
59 Vera-Román JM, Rubio L. Nonmembranous HER2/neu immunohistochemical staining. Appl Immunohistochem Mol Morphol 2003;11(04):364–365
60 Lu Y, Zhu S, Tong Y, et al. HER2-low status is not accurate in breast cancer core needle biopsy samples: an analysis of 5610 consecutive patients. Cancers (Basel) 2022;14(24):6200
61 Wolff AC, Somerfield MR, Dowsett M, et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO-College of American Pathologists Guideline Update. J Clin Oncol 2023;41 (22):3867–3872
62 Miglietta F, Griguolo G, Bottosso M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer 2021;7(01):137
63 Sajjadi E, Venetis K, Ivanova M, Fusco N. Improving HER2 testing reproducibility in HER2-low breast cancer. Cancer Drug Resist 2022;5(04):882–888
64 Sajjadi E, Guerini-Rocco E, De Camilli E, et al. Pathological identification of HER2-low breast cancer: tips, tricks, and troubleshooting for the optimal test. Front Mol Biosci 2023; 10:1176309
65 Casterá C, Bernet L. HER2 immunohistochemistry inter-observer reproducibility in 205 cases of invasive breast carcinoma additionally tested by ISH. Ann Diagn Pathol 2020;45:151451
66 Albagoush SA, Zubair M, Limaiem F. Tissue Evaluation for HER2 Tumor Marker. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Updated January 7, 2024. Accessed November 20, 2025 at: https://www.ncbi.nlm.nih.gov/books/NBK537134/
67 Standardization of Manual Method of Immunohistochemical Staining for Breast Cancer Biomarkers at Tertiary Cancer Care Center. An audit. Cureu. 2022;14(06):e25889
68 Hicks DG, Buscaglia B, Goda H, et al. A novel detection methodology for HER2 protein quantitation in formalin-fixed, paraffin embedded clinical samples using fluorescent nanoparticles: an analytical and clinical validation study. BMC Cancer 2018;18(01): 1266
69 Zhang H, Moisini I, Ajabnoor RM, Turner BM, Hicks DG. Applying the new guidelines of HER2 testing in breast cancer. Curr Oncol Rep 2020;22(05):51
70 Qaiser T, Mukherjee A, Reddy Pb C, et al. HER2 challenge contest: a detailed assessment of automated HER2 scoring algorithms in whole slide images of breast cancer tissues. Histopathology 2018; 72(02):227–238
71 Koopman T, Buikema HJ, Hollema H, de Bock GH, van der Vegt B. What is the added value of digital image analysis of HER2 immunohistochemistry in breast cancer in clinical practice? A study with multiple platforms. Histopathology 2019;74(06): 917–924
72 Frey P, Mamilos A, Minin E, et al. AI-based HER2-low IHC scoring in breast cancer across multiple sites, clones, and scanners. J Clin Oncol 2023;41(16, suppl):516

