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Review Article

International Expert Consensus Recommendations for HER2 Reporting in Breast Cancer: Focus on HER2-Low and Ultralow Categories

Emad A. Rakha[a,b,*], Puay Hoon Tan[c], Mieke R. Van Bockstal[d], Kimberly H. Allison[e], Edi Brogi[f], Grace Callagy[g], Gábor Cserni[h], Shabnam Jaffer[i], Maria Pia Foschini[j], Helenice Gobbi[k], Janina Kulka[l], Xiaoxian Li[m], Elena Provenzano[n], Abeer M. Shaaban[o], Gary M. Tse[p], Zsuzsanna Varga[q], Anne Vincent-Salomon[r], Rin Yamaguchi[s], Wentao Yang[t], Soha ElSheikh[u], Rohit Bhargava[v], Marina De Brot[w], Rita Canas-Marques[x], Caterina Marchiò[y,z], Madhuri V. Warren[aa], Carolien H.M. van Deurzen[bb], Yasmeen Mir[cc], Rahul Deb[dd], Hannah Wen[ee], Ian O. Ellis[a,ff], Stuart J. Schnitt[gg], Sarah E. Pinder[hh], Wendy Raymond[ii], Cecily Quinn[jj]; On behalf of the UK National Coordinating Committee of Breast Pathology, the Association of Breast Pathology, the European Working Group for Breast Screening Pathology and the International Society of Breast Pathology

[a] *Academic Unit for Translational Medical Sciences, School of Medicine, University of Nottingham, Nottingham, United Kingdom;* [b] *Department of Pathology, Hamad Medical Corporation, Doha, Qatar;* [c] *Luma Medical Centre, Royal Square Medical Centre, Singapore, Singapore;* [d] *Department of Pathology, Cliniques Universitaires Saint-Luc, Brussels, Belgium;* [e] *Department of Pathology, Stanford Medicine, Stanford, California;* [f] *Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York;* [g] *Discipline of Pathology, School of Medicine, University of Galway, Galway, Ireland;* [h] *Department of Pathology, University of Szeged, Szeged, Hungary & Bács-Kiskun County Teaching Hospital, Kecskemét, Hungary;* [i] *Northwell Health Lenox Hill Hospital, New York, New York;* [j] *Department of Biomedical and Neuromotor Sciences, University of Bologna, Anatomic Pathology at Bellaria Hospital, Bologna, Italy;* [k] *Discipline of Special Pathology, Institute of Health Sciences, Federal University of Triangulo Mineiro, Uberaba, Minas Gerais, Brazil;* [l] *Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Budapest and Eurofins Medserv Ltd., Budapest, Hungary;* [m] *Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia;* [n] *Department of Histopathology, Addenbrooke's Hospital and National Institutes of Health Cambridge Biomedical Research Centre, Cambridge, United Kingdom;* [o] *Cellular Pathology, Queen Elizabeth Hospital, Birmingham and Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom;* [p] *Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, HKSAR, China;* [q] *Department of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland;* [r] *Institut CURIE, Department of Diagnostic and Theranostic Medicine, Institute of Women's Cancer, Paris, France;* [s] *Breast Center/Department of Diagnostic Pathology, Nagasaki University Hospital, Nagasaki, Japan;* [t] *Department of Pathology, Fudan University Shanghai Cancer Center, China;* [u] *Research Department of Pathology, University College London, London, United Kingdom;* [v] *Department of Pathology, University of Pittsburgh, UPMC Magee-Womens Hospital, Pittsburgh, Pennsylvania;* [w] *Department of Anatomic Pathology, A.C.Camargo Cancer Center, St., São Paulo, Brazil;* [x] *Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;* [y] *Candiolo Cancer Institute, FPO-IRCCS, Candiolo (TO), Italy;* [z] *Department of Medical Sciences, University of Turin, Turin, Italy;* [aa] *Department of Cellular Pathology, Barts Health NHS Trust and Barts Cancer Institute, Queen Mary University of London, London, United Kingdom;* [bb] *Department of Pathology, Erasmus MC Cancer Institute, Rotterdam, the Netherlands;* [cc] *Pathology Department, Liverpool University Hospitals National Health Service Foundation Trust, Liverpool, United Kingdom;* [dd] *Pathology Department, University Hospitals of Derby & Burton, Royal Derby Hospital, Derby, United Kingdom;* [ee] *Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York;* [ff] *Department of Histopathology, City Hospital Campus, Nottingham University Hospitals, Nottingham, United Kingdom;* [gg] *Department of Pathology, Brigham and Women's Hospital and Harvard Medical School; Breast Oncology Program, Dana-Farber/Brigham Cancer Center, Boston, Massachusetts;* [hh] *School of Cancer & Pharmaceutical Sciences, King's College London and Guy's and St Thomas' Hospitals, London, United Kingdom;* [ii] *Department of Surgical Pathology Flinders Medical Centre, Flinders University and Clinpath Pathology, Adelaide, Australia;* [jj] *Irish National Breast Screening Programme and Department of Histopathology, St. Vincent's University Hospital, and School of Medicine, University College, Dublin, Ireland*

* Corresponding author.
E-mail addresses: emad.rakha@nottingham.ac.uk; emadrakha@yahoo.com (E.A. Rakha).

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ABSTRACT

The concept of "HER2-negative" breast cancer is evolving, with the recognition of HER2-low and HER2-ultralow subsets. These subsets are clinically relevant regarding treatment with the antibody-drug conjugate trastuzumab deruxtecan (T-DXd), which has shown survival benefit in patients with metastatic carcinoma with minimal HER2 protein expression that lack *HER2* gene amplification by in situ hybridization. In clinical trials using T-DXd, HER2-low was defined as an immunohistochemistry (IHC) score 1+ or an IHC score 2+ without *HER2* gene amplification. HER2-ultralow was defined as faint or barely perceptible, incomplete membrane staining in >0% to ≤10% of tumor cells (IHC score 0+/with membrane staining) and HER2-null as the complete absence of staining (IHC score 0/absent membrane staining). These results now necessitate more detailed evaluation and reporting of traditional "HER2-negative" results to identify patients with metastatic breast cancer who may benefit from T-DXd therapy. Both the US Food and Drug Administration and the European Medicines Agency have extended the regulatory approval of T-DXd to patients with metastatic breast cancer showing HER2-low or HER2-ultralow expressions. Updated clinical management guidelines now, therefore, incorporate the spectrum of HER2 results into treatment selection algorithms in the metastatic setting. To align histopathologic practice with these developments, the College of American Pathologists has issued a new biomarker-reporting template that recommends explicit distinction between IHC 0/absent membrane staining and IHC 0+/with membrane staining. Key concerns among pathologists include assay variability, scoring reproducibility, and quality assurance standards for accurately detecting such low levels of HER2 expression. This manuscript provides expert consensus, evidence-based practical recommendations for identifying and reporting tumors with HER2-low and HER2-ultralow expression. We emphasize standardized testing protocols, validated assays, robust internal and external controls, and focused training for pathologists. A universal structured pathology report is proposed to highlight the accurate distinction between IHC 0 (null), IHC 0+ (ultralow), and HER2-low expressions.

Introduction

HER2 is a membrane-bound tyrosine kinase receptor that dimerizes with other members of the epidermal growth factor receptor family.[1-3] Normal cells have 2 *HER2* gene copies and express low levels of HER2 protein, approximately 40,000 molecules per cell.[4,5] This baseline expression in normal cells influenced the calibration of HER2 immunohistochemical (IHC) assays to detect pathological overexpression associated with gene amplification. Conventional HER2-positive (IHC 3+ and/or *HER2* gene amplified by in situ hybridization [ISH]) breast cancer (BC) tumor cells may harbor up to 25-50 copies of *HER2*, resulting in a 40-fold to 100-fold increase in HER2 protein expression and as many as 2 million HER2 receptors on the cell surface.[4-6] HER2 protein overexpression presents a unique opportunity to utilize therapies that target the HER2 oncogenic pathways and, in turn, significantly improve clinical outcomes for patients with HER2-positive BC.[7-9]

Establishing the HER2 Testing Framework

The original HER2 IHC scoring system used in the trastuzumab clinical trials was designed to semiquantitatively assess membranous HER2 protein expression.[10,11] The scoring criteria were based on correlating membranous IHC staining intensity and completeness of membrane staining with *HER2* gene amplification status.[12-16] These studies demonstrated a strong association between protein overexpression and high *HER2* gene copy number, as well as a clinical benefit from trastuzumab in patients with HER2-positive BC. In the pivotal trastuzumab registration study, HER2 status was determined centrally by IHC with the murine monoclonal antibody 4D5, the prototype antibody from which trastuzumab was humanized.[10,11] The 4D5 manual IHC assay (developed jointly by Genentech and Dako) used the now-familiar 0, 1+, 2+, and 3+ scoring system; patients with tumors scoring 2+ or 3+ were deemed "HER2-overexpressing" and eligible for enrolment.[8] The investigational 4D5 method was later commercialized, with minor technical refinements, as the HercepTest (Dako/Agilent) and became the first US Food and Drug Administration (FDA)-approved companion diagnostic IHC kit for trastuzumab. The first large, prospective trials to mandate reflex ISH testing on tumors with a 2+ IHC score to determine *HER2* gene status were the paired US adjuvant studies NSABP B-31 and NCCTG N9831.[11] These established the now-standard practice of using reflex ISH to adjudicate equivocal (2+) IHC results before offering trastuzumab, a strategy subsequently adopted by other landmark studies, including HERA and BCIRG 006.[17,18] Patients with tumors with HER2 IHC scores 0 or 1+ were not enrolled in these trials because these tumors rarely exhibited *HER2* gene amplification, and no clinical benefit was anticipated from HER2-targeted monoclonal antibodies.[9,19] Consequently, IHC scores of 0 and 1+ were historically both considered "HER2-negative," as the distinction between 0 and 1+ had no clinical implications. Tumors with a 2+ IHC score and ISH-negative results were also classified as HER2-negative. The efficacy of anti-HER2 monoclonal antibody therapy in HER2-positive BC has been confirmed through multiple additional clinical trials, leading to expanded approval for additional HER2-targeted therapies.[20-23]

The HercepTest HER2 staining system from Dako (Agilent) and its accompanying HER2 scoring criteria were long regarded as the "gold standard" for HER2 testing in BC. Subsequently, additional commercial assays became available, most notably, the PATHWAY anti-HER2/neu (Ventana/Roche), which employs the rabbit monoclonal antibody 4B5, and the Bond Oracle HER2 IHC System (Leica Biosystems), which uses the mouse monoclonal antibody CB11. Multiple studies have demonstrated good overall

concordance; however, some interassay variability and occasional discrepant results have been reported.[24,25] Nevertheless, the primary focus was on accurately identifying HER2-positive tumors, with less emphasis on subclassifying HER2-negative BC.

Historical Overview of HER2 Scoring Guidelines

Recognizing the critical importance of test reliability and reproducibility, guidelines were published to provide recommendations on quality measures aimed at ensuring HER2 testing accuracy.[14,26-29] These initially aligned with the original scoring criteria established for the Dako HercepTest. The first national guidelines were issued in the United Kingdom in 2000[13] and updated in 2004[29] and 2015,[14] introducing a combined category of "HER2-negative" that encompassed IHC scores of 0 and 1+. In 2007, the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) jointly published the first ASCO/CAP HER2 testing guidelines,[30] which also included both IHC scores of 0 and 1+ as "HER2-negative." Applying these guidelines, there was a significant correlation between HER2 IHC scores and mean *HER2* copy number.[16,31] However, there have been further refinements of the ASCO/CAP IHC/ISH scoring definitions in 2013,[27] 2018,[28] and, most recently, 2023,[26] now including rare and ambiguous staining patterns that continue to pose occasional diagnostic challenges.

Novel Antibody-Drug Conjugate Trastuzumab Deruxtecan Is Effective in Patients With Metastatic Breast Cancer With Low Levels of HER2 Protein: HER2-Low and HER2-Ultralow

The novel HER2-targeted antibody-drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) has demonstrated clinical efficacy in patients with metastatic BC with HER2 IHC score 1+ or 2+/ISH-negative tumors (designated "HER2-low" in the trials DESTINY-Breast04 and 06)[32,33] and in hormone receptor (HR)-positive tumors with ≤10% faint or barely perceptible, incomplete, HER2 membrane staining considered score 0+ but with some membrane staining (designated "ultralow" in the trial DESTINY-Breast06).[33] These trials have expanded the therapeutic options for a substantial subset of BC patients and established the use of HER2-low[9,34,35] and HER2-ultralow[33] terminologies in clinical practice to refer to treatment-eligible metastatic BC patients. T-DXd has now been approved for BC patients with metastatic or unresectable HER2-low tumors in many countries.[36,37]

Multiple studies have aimed to clarify if there is any additional biological or clinical significance to HER2-low BC as defined in these clinical trials or if this terminology is only relevant to the trial entry criteria used to grant drug approval.[7,38-43] HER2-low status is seen in approximately 40% to 60% of all BCs.[35] Studies have demonstrated that it constitutes a heterogeneous group with variable clinical, histopathological, and molecular features,[39,44-49] and it is unclear if this is a distinct biological entity, which appears to be influenced by the HR status.[50-52] Notably, the clinical benefit of T-DXd in patients with HER2-low BC is attributed to the presence of HER2 protein on the tumor cell surface, rather than to HER2-driven oncogenic signaling. Unlike trastuzumab, which targets HER2-mediated pathways and is not effective in HER2-low BC, T-DXd targets surface HER2 protein to internalize its cytotoxic "payload" (topoisomerase I inhibitor deruxtecan).[19,32,33,53] Thus, the response to T-DXd is likely to be independent of the biological activity of HER2, and alternative oncogenic pathways may govern the behavior of these tumors.[54,55] The T-DXd payload is also membrane-permeable; hence, it can diffuse into neighboring cells, allowing a "bystander effect" in HER2-negative cells.[56] Evolving in vitro evidence also suggests that some T-DXd activity can occur independently of HER2 engagement and internalization.[57]

Therapeutic Implications of HER2-Low and Ultralow Expression

The findings from DESTINY-Breast06 (DB-06)[33] have raised debate over whether the presence of any HER2 membrane protein, even below IHC detection thresholds, may enable T-DXd activity if a subset of tumor cells expresses sufficient HER2 molecules for internalization. Commercially available HER2 IHC assays are optimized to detect membranous HER2 overexpression above the physiological baseline seen in normal epithelial cells, which implies that some or all HER2-null cases (IHC score 0) may have levels of membranous HER2 protein below the level detectable by such assays.

In the DB-01-03 trials,[58,59] which enrolled HER2-positive BC patients, objective response rates exceeded 60%. By contrast, DB-04[32] and DB-06[33] included only patients with HER2-low and HER2-ultralow tumors and reported lower response rates (~30% and ~20%, respectively). These differences are thought to be related to the ability of the monoclonal antibody in the T-DXd complex (trastuzumab) to be more effective in HER2-driven BC once it is released from the ADC complex.[57] Interestingly, differences in response rates to T-DXd between IHC 1+ and 2+ in the HER2-low cohorts were not demonstrated in DB-04. This is in conjunction with the DB-06 data showing that T-DXd is equally effective in the treatment of tumors with <10% faint, incomplete membrane staining (ultralow), supporting the notion that any amount of HER2 protein may be used to deliver a payload that can have far-reaching effects. For example, the phase 2 DAISY trial assessed the efficacy of T-DXd in patients with HER2-overexpressing (cohort 1), HER2-low (cohort 2), and HER2-non−expressing (cohort 3) metastatic BC and demonstrated objective response rates of 70.6% in cohort 1, 37.5% in cohort 2, and even 29.7% in cohort 3.[60]

Evolving Guidelines for HER2-Low and Ultralow

National guideline recommendations have varied in their approach to defining and classifying HER2-low BC, and none have yet formally addressed the ultralow category.[14,26] The 2023 ASCO/CAP focused update[26] did not endorse HER2-low as a reporting category, noting that HER2 IHC 0 tumors were excluded from the DB-04 trial; hence, the predictive power of a HER2-low vs HER2-null status was untested.[32] In addition, the discrete HER2 results (IHC 1+ or 2+/ISH-negative) were sufficient to identify patients who met trial eligibility using the guidelines-based criteria in DB-04.[26] In DB-06, HER2 ultralow tumors were also defined based on the ASCO/CAP criteria, describing the 2 staining patterns of IHC score 0 cases. However, because these 2 patterns are typically reported under the same IHC score of 0, identification of ultralow results with <10% faint, incomplete membrane staining created a new reporting issue.

Both the US FDA[61] and the European Medicines Agency have now extended the regulatory approval of T-DXd to patients with HER2-low tumors and, when HR-positive, to patients with HER2-ultralow unresectable or metastatic BC as defined in the DB-04[32] and 06[33] trials. Reflecting these approvals, the National Comprehensive Cancer Network[36] and an expert consensus from

the European Society of Medical Oncology[62] incorporated T-DXd into treatment pathways in the metastatic BC setting based on the specific details of the HER2 IHC results.

To consolidate reporting, the CAP has released a new biomarker-reporting template (effective March 2025)[63] that recommends recording 4 discrete IHC HER2-negative strata, 0, 0+, 1+, and 2+/ISH-negative, with the option to add a comment as to how "HER2-low" and "HER2-ultralow" were defined in the relevant clinical trials. Comprehensive guidance for pathologists in testing methodology and interpretation of low-level HER2 IHC expression remains an urgent priority to ensure diagnostic consistency and optimal patient selection for ADCs.

Challenges in Accurate Classification of HER2-Low and Ultralow

Although HER2-low and ultralow BCs have gained clinical momentum, concerns remain regarding the reliability of the current HER2 testing methodology in accurately identifying these categories. Studies have highlighted low interobserver concordance among pathologists in identifying HER2-low and ultralow tumors using current scoring criteria.[64-71] In the DB-06 trial,[33] central reevaluation of HR-positive BC classified locally as IHC 0, revealed that 35% were true HER2-null (0), 40% were ultralow (0+), and 24% were reclassified as IHC 1+. This illustrates the prevalence of HER2-low and ultralow tumors, collectively representing more than half of BC.[72] It also highlights the challenges of consistent classification at the lower end of the HER2 expression spectrum, and the need for pathologist training, standardization of interpretation, and consideration of alternative tools such as artificial intelligence (AI) algorithms to more reproducibly define low levels of HER2 protein expression.

Sensitivity also varies among commercial detection kits, particularly at very low HER2 expression levels, and some interlaboratory variation is inevitable. Whether such discrepancies translate into clinically meaningful differences in response to T-DXd remains unknown. Consequently, laboratories should implement measures to maximize staining consistency and ensure optimal performance of the existing approved and validated assays. As current regulatory approvals for T-DXd are based on the performance of this existing HER2 assay, there is no evidence to suggest that changing to newer, more sensitive assays (should they become available) would improve the identification of patients likely to demonstrate therapeutic response to ADCs. In addition, despite the limitations of the existing assay and methodology, it remains the only widely available and validated method. Newer approaches, such as quantitative immunoassays or RNA-based platforms, may provide greater accuracy in the future, but until these are standardized, validated, and proven in clinical trials, the practical strategy is to continue with current assays, supported by rigorous training, audit, and quality control to maximize reproducibility at the low expression range.

Finally, selecting the most appropriate tumor sample(s) to be examined also poses practical challenges. There is evidence that HER2 IHC expression in non-HER2 amplified BC is variable between samples and with disease progression. Although in 1 series, most tumors initially scored as HER2-low retained that status on repeat biopsy after progression,[73] other studies reported a 14% to 57% change from IHC 0/0+ to HER2-low and a 23% to 25% shift in the opposite direction in residual disease after neoadjuvant therapy.[74,75] Subtle differences in assay performance and tissue sample handling may account for some of these differences. Spatial and temporal heterogeneity in HER2 expression[76] further complicates the identification of negative tumors and increases the risk of false-negative results. These data highlight the need for reliable HER2 testing methods for low levels of protein expression and reinforce the importance of retesting when treatment decisions hinge on precise categorization.

Framework for HER2-Low/Ultralow Testing and Interpretation

The issues of testing and interpretation reliability highlight the urgent need for updated quality assurance protocols to ensure consistent and reproducible HER2 assessment across laboratories.

Based on a comprehensive literature review and recent recommendations for refining HER2 assessment, the proposed updated HER2 scoring algorithm is summarized in Table 1 and Figure 1. This synthesis incorporates the inclusion criteria of 2 pivotal T-DXd trials (DB-04 and 06),[32,33] alongside current international guidelines and best practice recommendations.[77-79] In addition, certain rare and unusual HER2 staining patterns, not explicitly addressed in the existing ASCO/CAP guidelines, have been incorporated.[55] Their inclusion aims to help improve diagnostic consistency and address interpretative challenges, rather than introducing new diagnostic entities.

The recommendations outlined below provide an updated framework for HER2 evaluation in routine pathology practice, with emphasis on the accurate identification and reporting of HER2-low and HER2-ultralow BC. To ensure reproducibility and clinical utility, strategies for optimizing HER2 testing must include stringent quality control protocols and harmonized pathology reporting, thereby supporting informed and consistent clinical decision-making (Table 2).

Preanalytical Considerations

These measures are the same as those currently used to optimize the detection of HER2-positive BC, as previously recommended.[14,26-29] Delays in fixation should be minimized (cold ischemia time <1 hour), as this may reduce HER2 antigen detection and lead to false-negative results, particularly in HER2-ultralow tumors (IHC score 0+), which may appear HER2-null (IHC score 0). The DB-06 trial[33] criteria specified specimen fixation in 10% neutral-buffered formalin (recommended fixation time 6-72 hours to a maximum of 96 hours[80-82]). Ideally, HER2 should be assessed on freshly/recently cut sections from a properly stored formalin-fixed paraffin-embedded (FFPE) sample from the most recent specimen. Sections >6 weeks may show reduced antigenicity. Control sections should ideally be cut simultaneously to ensure optimal staining comparability. Additionally, an on-slide external control with different levels of HER2 expression (eg, "in-house" tumor samples, commercially available cell lines, or newer calibrators such as Boston Cell Standards) is highly recommended. Although long-term storage of precut control sections (ie, >6 weeks) is discouraged, they may be acceptable if validated by the laboratory. This includes appropriate storage, such as refrigeration, and verification of stability over the intended period of use. In metastatic disease, a newly acquired biopsy specimen should be obtained if clinically feasible.

In the context of HER2-low and HER2-ultralow testing for clinical decision-making, multiple tumor samples are often available, including the primary invasive carcinoma, metastases (including lymph node deposits), and post-treatment

Table 1
Clinically relevant HER2 scoring categories with proposed additional guidance for rare and unusual staining patterns[c]

HER2 IHC score	Clinical category	Definition
Score 0/absent membrane staining	HER2-null	No membrane staining of invasive tumor cells
Score 0+/with membrane staining	HER2-ultralow	Faint/barely perceptible, incomplete, membrane staining in ≤10% of invasive tumor cells. This category may also be used for the following unusual staining patterns: 1. Faint/barely perceptible membrane staining in ≤10% of invasive tumor cells, regardless of the circumferential nature of membrane staining.[a,b] 2. Weak, incomplete, membrane staining in ≤10% of invasive tumor cells.[c] The lower level of positivity is not defined by 1% but includes >0%-10%.[33]
Score 1+	HER2-low	Faint/barely perceptible, incomplete, membrane staining in >10% of invasive tumor cells. This category may also be used for the following unusual staining patterns: 1. Weak, complete, membrane staining in ≤10% of invasive tumor cells. 2. Weak, incomplete, membrane staining in >10% of invasive tumor cells. 3. Moderate, incomplete, membrane staining in ≤10% of invasive tumor cells.
Score 2+	HER2-low if ISH-negative HER2-positive if ISH positive	Weak to moderate complete membrane staining in >10% of invasive tumor cells.[c] This category may also be used for the following unusual staining patterns with a recommendation for reflex ISH testing: 1. Moderate, complete, membrane staining in any percentage of invasive tumor cells. 2. Moderate to strong, incomplete, membrane staining in >10% of invasive tumor cells. 3. Strong, complete, membrane staining in ≤10% of invasive tumor cells. 4. Abundant cytoplasmic staining present, obscuring the membrane stain intensity.
Score 3+	HER2-positive	Strong, complete, membrane staining in >10% of invasive tumor cells.[c]

IHC, immunohistochemistry; ISH, in situ hybridization.

[a] Artefactual HER2 staining on the apical/luminal surface of glandular epithelial cells lining tubules or acini or staining limited to the periphery of cell clusters without an intercellular membrane pattern represents a nonspecific IHC pattern that may mimic true membranous positivity, particularly in the context of HER2-low or HER2-ultralow scoring. In addition, edge artifacts should not be scored, especially in small biopsies or poorly preserved tissue sections. They may be attributed to suboptimal fixation, drying artifacts, antibody diffusion, or section thickness and folding. In tumors with edge artifacts or if the control of 1+ shows suboptimal staining, repeat staining is recommended to distinguish between HER2-null and HER-ultralow.

[b] Rare staining patterns not addressed in the American Society of Clinical Oncology and the College of American Pathologists guidelines (may occasionally be encountered in clinical practice). These have been described in the updated United Kingdom guidelines and in other international recommendations and studies.[55,77-79] Their inclusion here aims to enhance the consistency of HER2 reporting, rather than to introduce new scoring categories. These patterns should be interpreted with caution and, if in doubt, repeat testing on the same or an alternative tissue block/sample and/or ISH testing is advised.

[c] Distinguishing between moderate and strong staining intensity can be subjective. Comparison with appropriate positive controls, such as known HER2 3+ cases, is essential to identify true strong intensity. Any staining weaker than this benchmark should be regarded as moderate. In ambiguous situations, whether due to uncertainty about intensity (moderate versus strong) or the percentage of stained invasive tumor cells, ISH testing can help to clarify the HER2 status. As a general guide, membrane staining visible at ×10 objective magnification typically reflects weak intensity, as faint staining usually becomes discernible at ×20 and is clearly visible at ×40 magnification.

specimens. HER2-low expression is highly heterogeneous with reported discordance between core biopsies and surgical excisions,[83] as well as between primary tumors and locoregional recurrences or distant metastases.[38] Discordance has also been observed among different metastatic sites and within the same site at different time points.[84,85] According to current guidance, if any tumor sample during a clinical course is scored as HER2-low, the patient is eligible for consideration for T-DXd therapy. The DB trials permitted the inclusion of all available sample types, allowing pathologists to select the most appropriate for testing. For patients enrolled in DB-04, the efficacy of T-DXd, compared with the physician's choice therapy, was consistent regardless of sample characteristics, including type, collection date, tumor location, and specimen type.[86] Consequently, multiple samples (eg, 2-3) can be tested, particularly if the initial specimen is HER2-null, and the patient is otherwise eligible for treatment, as determined by the oncologist (Supplementary Table S1). Fine-needle aspiration biopsies and decalcified bone metastasis specimens are not suitable for the reliable assessment of HER2-low or HER2-ultralow status. If no alternative tissue is available, testing of these specimens may be performed; however, the absence of staining may not be reliable, and background staining in cell block material is likely to prohibit accurate assessment of IHC 0+ or 1+. Routine reexamination of archived HER2 IHC-stained slides (eg, >5 years) that were originally reported as HER2 0 is also discouraged because immunoreactivity may fade over time, and a genuine HER2-ultralow case may be misread as HER2-null.

Recommendations:

1. *Fixation*: Use 10% neutral-buffered formalin with cold ischemic time <1 hour, and fixation time of 6 to 72 hours, up to a maximum of 96 hours.
2. *Sample selection*: Use the most recent FFPE tissue blocks.
3. *Repeat testing*: Generally unnecessary. However, if the original specimen was reported as HER2-0 and did not distinguish between HER2-null and HER2-ultralow, and the patient could benefit from T-DXd as determined by the oncologists, consider rescoring the most recently stained slide (if <5 years) (with the proviso that reactivity may fade over time) or retesting on the archival tumor block. If uncertainty persists, test additional blocks, from the same or alternative sites.

Analytical Considerations

In the T-DXd development trials,[32,33] 4-μm-thick sections from FFPE blocks were used, unstained slides were stored at 2°C to 8°C or at room temperature (15°C-25°C), and staining utilized the VENTANA HER2 (4B5) assay. In the DB-04 and -06 trials,[32,33] the HER2 test was repeated, or tested by ISH; if controls were not as expected, the sample had strong membrane staining of normal breast ducts (internal control), or if staining artifacts interfered with interpretation. Only intact/viable tumor cells were considered for the interpretation of staining, as necrotic or degenerate

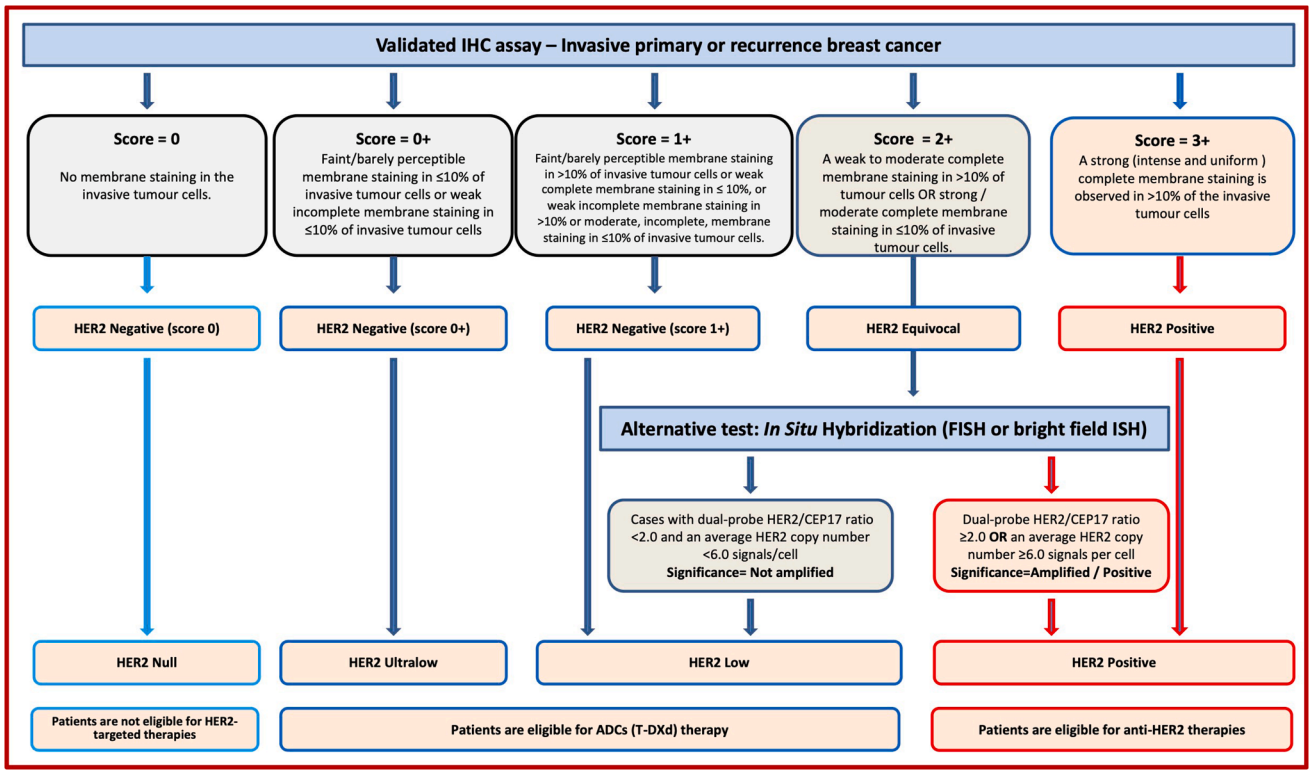

Figure 1.
Proposal for HER2 scoring algorithm (modified from Rakha et al[77]). Current United Kingdom guidelines[14,77] consider the ISH test positive if the dual-probe *HER2/CEP17* ratio is ≥2.0 or the average *HER2* copy number is ≥6.0 signals per cell. In contrast, the American Society of Clinical Oncology and the College of American Pathologists guidelines[26,28] define positivity as a dual-probe *HER2/CEP17* ratio ≥2.0 (and with an average HER2 copy number ≥4.0) or an average *HER2* copy number ≥6.0 signals per cell. ADCs, antibody-drug conjugates; FISH, fluorescent in situ hybridization; ISH, in situ hybridization; T-DXd, trastuzumab deruxtecan.

cells may show nonspecific reactivity. Background staining was defined as nonspecific staining.

Antibody Selection

Although the DB-06 trials centralized HER2 testing using the FDA-approved VENTANA HER2 (4B5) assay with the ultraView detection system to ensure consistency, other assays were employed at local laboratories during the initial screening phase.[28,32] In a recent study, 14 laboratories employed the VENTANA HER2 (4B5) assay with ultraView detection and demonstrated minimal interlaboratory variation in results.[87] The Nordic Immunohistochemical Quality Control (NordiQC) proficiency testing program[25] showed considerable harmonization of HER2 IHC protocols: >78% of participating laboratories now use FDA- or CE-IVD-approved assays rather than laboratory-developed tests, and the overall pass rate for HER2 IHC assessment reached 90%. Data from the UK HER2-low National External Quality Assessment Scheme (NEQAS) showed that 87.3% of laboratories used the 4B5 assay (Ventana), which achieved the highest pass rate (73.3%) among participating centers.[88]

Current evidence indicates that only the specific, FDA-approved, companion diagnostic assay reproduces the diagnostic and therapeutic outcomes reported in the DB-04 trial.[32] Roche Tissue Diagnostics has since released an updated FDA-approved protocol that further optimizes the PATHWAY anti-HER2 (4B5) assay for detecting HER2-low and HER2-ultralow

Table 2
Summary of key steps for HER2-low and ultralow quality assurance

Quality measure
1. Standardize tissue handling and fixation.
2. Use validated HER2 IHC assays.
3. Use appropriate internal and external quality controls with a range of expression (0, 1+, 2+, 3+).
4. Validate assay performance in HER2-low/ultralow cohorts as well as in HER2-positive.
5. Ensure reports use the 5 clinically relevant HER2 IHC score categories, including score 0/absent membrane staining, 0+/with membrane staining, 1+, 2+, and 3+.
6. Evaluate HER2 IHC 2+ tumors with ISH.
7. Use standardized reporting (such as CAP synoptic templates) and monitor for discrepancies.
8. Ensure pathologists undergo training for HER2 assessment, particularly to identify HER2-low and ultralow cases.
9. Participate in EQA programs for HER2 testing.
10. Ensure good communication between oncologists and pathologists for the identification of patients with HER2-low and ultralow categories.
11. Regularly audit HER2 results and compare with reference standards.
12. Double report on ambiguous and challenging cases.

CAP, College of American Pathologists; EQA, external quality assurance; IHC, immunohistochemistry; ISH, in situ hybridization.

tumors. To maintain performance, laboratories must follow the locked FDA protocol, and previous user-adjustable parameters, such as CC1 cell conditioning and primary-antibody incubation, are now fixed, with the latter set at 12 minutes. Compliance with this locked protocol is mandatory for determining T-DXd eligibility. Several alternative HER2 IHC assays may demonstrate greater analytical sensitivity than the Ventana 4B5/ultraView combination,[32] but as no clinical outcome data exist for other HER2 IHC platforms, it is uncertain whether achieving higher analytical sensitivity yields equivalent patient selection for T-DXd. Therefore, comparisons should be limited to analytical performance rather than presumed clinical benefit. Laboratories using alternative assays should ideally formally cross-validate their results against the Ventana PATHWAY anti-HER2 (4B5) assay platform and participate in technical HER2-low and -ultralow external quality assurance (EQA) schemes to ensure continued compliance.

Although newer quantitative assays, such as the HERmark BC assay,[89,90] immunofluorescence-based automated quantitative analysis methods,[91,92] quantitative IHC methods,[93] and assessment of *HER2* messenger RNA expression levels[94-96] are being evaluated, as stated above, these have not been validated for use in the clinical setting. Therefore, existing efforts are best directed toward standardizing current assays, adhering to guideline recommendations for HER2 testing[14,27,28,30] and improving pathologist concordance in categorizing HER2 status.[68,97] Assays should be appropriately validated in line with recognized international guidelines, which require demonstration of assay accuracy, reproducibility, and concordance with an established reference standard before clinical implementation.[14,27,30,37]

Recommendations:

1. *Assay selection*: Validated, guideline-endorsed assays are preferred. Currently, VENTANA HER2 (4B5) with ultraView locked protocol is advised. Alternative validated HER2 assays can be used, but should be appropriately validated.
2. *Slide preparation and storage*: Use 4 μm-thick FFPE sections; stain within 6 weeks and store unstained slides as per assay guidelines.

Quality Control

Quality control measures include internal quality control and quality assurance (QA) procedures, in addition to participation in external proficiency testing and accreditation by a valid accrediting agency.

HER2 Staining Controls

Both internal and external controls are required in every IHC run. Internal negative elements, such as benign epithelial cells and stroma, should be evaluated on each slide when present. The FDA premarket approval for the Ventana PATHWAY anti-HER2 (4B5) assay, and the locked protocol subsequently adopted for T-DXd eligibility, specify that a validated negative-control tissue section (HER2 0) must be assessed in parallel with each run. Reagent (isotype) controls or buffer-only controls are required whenever a new lot or significant change is introduced.

HER2 controls should cover the full dynamic range of expression. Existing control methods primarily focus on identifying HER2-positive tumors, rather than distinguishing between 1+, 0+, and 0 scores. However, the HER2-low and -ultralow categories differ from both HER2-positive and HER2-null tumors in terms of staining characteristics and control requirements. To address this, laboratories should supplement traditional HER2-positive and HER2-negative controls with additional controls that include intermediate and low expression levels.

Cell line controls can better capture the faint, heterogeneous staining typical of HER2-low and -ultralow tumors and offer consistent and homogeneous staining due to their derivation from clonal populations with stable protein expression. However, because such lines are scarce, most laboratories rely on well-characterized tissue sections. When selecting these, it is important to include the full spectrum of staining intensities, strong, moderate, weak, and faint; the range is more informative than the percentage of positive cells. The cutting of deeper levels from a control block can alter the proportion of cells exhibiting each pattern; hence, controls containing a relatively high proportion of the target intensity are preferred.

The following controls can be used:

HER2-positive controls: These remain unchanged and include HER2 IHC 3+ cell lines (eg, SK-BR-3 and BT-474) and/or HER2 IHC 3+ BC tissue. Although minimal biological variation in IHC profiles may occur between cell line batches, such differences generally fall within acceptable levels and should not impact assay performance.

HER2-null controls: These include HER2 IHC 0 cell lines (eg, MDA-MB-231) or HER2-null BC tissue samples. The MCF-7 cell line is generally considered HER2-null (IHC 0), with a normal *HER2* gene copy number and minimal HER2 protein expression,[98] making it a widely accepted negative control. However, MCF-7 cells can occasionally exhibit low-level HER2 expression (IHC 1+), likely due to variability in culture conditions or subclonal heterogeneity.

HER2-low controls: These include the use of FFPE tissue blocks from BC confirmed as HER2-low IHC 1+ or 2+ (expressing moderate, weak, or faint staining pattern) or validated HER2-low cell lines. HER2 IHC 1+ cell lines can be used as reference material to assess staining pattern and technical performance, provided the percentage of staining is carefully interpreted in the context of invasive tumor cell distribution. Few cell lines have been identified as HER2-low, and most are not widely available. The T-47D cell line has been reported to exhibit HER2-low features in some studies. ZR-75-1 is a luminal BC cell line sometimes reported to display HER2 IHC 1+ or 2+ staining under specific conditions. A feature of the MDA-MB-175 1+ control cell line is a distinct growth pattern in which the cells form clusters with a continuous luminal brush border region. This should not be included in the HER2 staining evaluation nor the dot-like immunostaining of the Golgi region in the cytoplasm. It is the faint/barely perceptible membrane staining in any percentage of tumor cells that represents HER2 expression.[99]

HER2-ultralow controls: Currently, no dedicated cell lines are available to serve as staining controls for the HER2-ultralow category. Therefore, tumor tissue with a relatively high proportion of faint membrane staining should be used until such appropriate cell lines are available.

If cell lines are unaffordable or unavailable, control tissues with established HER2 expression levels can serve as suitable alternatives. Nonneoplastic epithelial cells within the section serve as valuable internal controls. Occasional weak basolateral staining in normal lobules is allowable, but staining that exceeds weak intensity or shows a complete membranous circumferential pattern should be regarded as overstaining, usually reflecting technical artifact or suboptimal tissue processing. Metaplastic apocrine cells may show cytoplasmic reactivity.

Batch-to-batch reproducibility of IHC reagents and antibodies must also be ensured to maintain staining consistency and diagnostic accuracy.

A recent, robust, and quantitative introduction for assessing IHC assay performance is the development of IHC calibrators.[87,100] These are composed of coated glass microbeads specifically designed to quantify key analytical parameters such as analytical sensitivity, lower limit of detection, and dynamic range. By standardizing these metrics, calibrators help explain interassay variability and differences in HER2 positivity rates across platforms.

Recommendations:

1. Use validated controls: Include internal and external HER2 controls in every IHC run covering a full range of expression (0, 1+, 2+, and 3+); use cell lines or tissue samples; on-slide external controls are recommended.
2. Maintain assay consistency: Ensure batch-to-batch reproducibility of reagents; consider deploying independent IHC calibrators whenever a new antibody or detection system lot is introduced or there are unexplained staining shifts. Any batch-related discrepancies should be reported to the relevant national EQA provider. This creates a central record to facilitate rapid troubleshooting and consistency across laboratories.

Quality Assurance Programs

Laboratories should participate in technical EQA programs, such as those organized by the UK NEQAS, CAP, NordiQC, or Royal College of Pathologists of Australasia Quality Assurance Programs, which provide interlaboratory comparison and critical performance feedback. Participation in EQA schemes that specifically assess HER2-low and ultralow IHC staining is particularly valuable, given the interpretative challenges at low expression levels. In parallel, regular internal quality assessments should be conducted. These may include the reevaluation of a subset of HER2 0, 0+, and 1+, as well as 2+ and 3+ cases, to verify staining consistency. Such practices enhance the accuracy, reliability, and reproducibility of HER2 staining in routine clinical diagnostics.

In addition to laboratory-based EQA, pathologists should be encouraged to participate in interpretative EQA schemes focused on HER2 scoring. Such schemes enhance diagnostic consistency, particularly in overlapping categories. In regions where these programs are not yet established and access to international initiatives is costly, national pathology societies, regulatory authorities, or EQA providers should take the lead in developing dedicated initiatives to support education, standardization, and ongoing quality improvement in the interpretation of IHC-based companion diagnostics such as HER2.

Internal Review

Laboratories should routinely monitor and review HER2 testing and the identification/reporting of HER2-low and ultralow categories and HER2-null tumors.[64-71] Departments should update testing protocols and pathologist training to reflect the new HER2-low and ultralow clinical guidelines. Pathologists' training should include structured educational updates, such as workshops and review of annotated case sets, with particular emphasis on challenging or indeterminate cases alongside classical HER2-low and ultralow examples. Resources include the online educational module (https://pathogate.net/her2-low), supported by the International Quality Network for Pathology and UK NEQAS. Training should also be reinforced by internal teaching sessions, departmental case-based discussions to enhance diagnostic consistency, and access to consensus guidelines with illustrative examples.

Case-based reviews may be undertaken either in real time (for difficult or indeterminate cases, before report sign-out) or retrospectively as part of routine audit exercises. Regular concordance checks should be performed within each pathology group or department, with particular focus on cases at the low/ultralow range. These can be organized as periodic double-reporting or group review exercises, during which scores are compared, discrepancies documented, and corrective actions implemented where necessary. Importantly, discrepancies refer to differences between the original and reviewed scoring categories, not to variation between specimens or tumor blocks, which reflects true intratumoural heterogeneity. The most effective approach combines both strategies: real time double review of challenging cases and retrospective group review of selected cases to enhance consistency across the pathologist team.

Recommendations:

1. *EQA participation*: Engage in technical and interpretative EQA schemes, especially for HER2-low/ultralow categories, and conduct internal audits/reviews to ensure consistency and concordance.
2. *Continuous improvement*: Update protocols, interpretation criteria, and training in line with evolving HER2-low/ultralow guidelines. Audit HER2 scoring discrepancies.

Postanalytical Considerations

HER2 Immunohistochemistry Interpretation and Scoring

Pathologists performing HER2 testing should be appropriately trained and experienced to ensure accurate interpretation of the IHC preparations. Scoring by 2 pathologists may further enhance diagnostic accuracy, particularly in indeterminate or challenging cases, as advocated by the ASCO/CAP 2023 best practice update.[101] The HER2 IHC score is assigned based on the extent, intensity, and degree of circumference involvement (complete or incomplete) of membranous HER2-specific staining, as in the ASCO/CAP reporting algorithm.[28] Cytoplasmic granular staining, reflecting mitochondrial localization, is frequently observed in carcinomas with apocrine differentiation. This, and nuclear staining, suggestive of nonspecific translocation, are not predictive of response to T-DXd and should not be scored.

In the 2 T-DXd trials, indeterminate cases were evaluated at ×40 objective magnification or higher to distinguish between IHC scores of 0, 0+, 1+, and 2+.[32,33] In routine practice, this "magnification rule"[77,102] for grading HER2 IHC intensity can be helpful. Strong intensity staining is visible at very low magnification and should closely match the external 3+ control slide. Moderate intensity staining may also be visible at low magnification and is best distinguished from strong staining by direct comparison to the 3+ control. Weak intensity staining is appreciable with a ×20 objective magnification and may occasionally be discerned at ×10. Faint staining is barely perceptible, best seen at ×40 objective magnification, and not seen at lower magnifications. This stepwise approach, progressing systematically from low to high magnification, enhances discrimination between

intensity levels.[102] Circumferentiality and the percentage of stained tumor cells are assessed separately at appropriate magnification.

In the trials, tumors with heterogeneous staining, where different regions of the tumor displayed varying staining intensities, the relative proportions of each intensity level were visually estimated. The final diagnostic score was assigned according to the highest scoring category, with a comment on the percentage of stained cells.[32,33]

Clear, concise reporting is essential. Each report should state the HER2 score (0, 0+, 1+, 2+/ISH results, or 3+), the methodology, including antibody clone or ISH probe, platform, and protocol. Optionally, a brief narrative may be added describing the staining pattern, intensity, and the approximate percentage of tumor cells stained for HER2. Where appropriate, the CAP-endorsed optional comment that outlines the clinical relevance of low-level HER2 expression and defines the HER2-low and HER2-ultralow categories used in recent clinical trials can be included.

For ISH, assessment of 20 to 60 invasive tumor cells is generally adequate for reliable categorization. In contrast, adjudicating HER2-low vs HER2-ultralow status relies on IHC; practical experience suggests that examining at least 100 invasive carcinoma cells improves the ability to distinguish between IHC 0, 0+, and 1+. If only very scant tumor cells are available, and no membranous staining is seen, the sample should be reported as "HER2 uncertain, as insufficient tumor cells are present for accurate assessment," with rebiopsy recommended if T-DXD therapy is being considered. If unequivocal membranous staining is present, an indication that "although only scant tumor cells are present, the score is at least ……..(*with relevant score*)" (Fig. 2).

Recommendations:

1. HER2 scoring should be performed by trained pathologists; dual scoring (eg, by 2 pathologists) should be considered for marginal cases. Clearly state the HER2 score, the interpreted category, and the method. Comments on the staining pattern, intensity, and approximate percentage of positive tumor cells are optional. If there are distinct clones of HER2-positive and HER2-negative tumor cells, this should be noted. Exclude necrotic areas, foci of carcinoma in situ, and artifactual staining.
2. Ensure adequacy of the sample. If only scant tumor cells are available and no membranous staining is seen, the sample should be reported as "HER2 uncertain, as insufficient tumor cells are present for accurate assessment," with rebiopsy recommended if T-DXd therapy is being considered.

Other Quality Measures

Pathologist Training and Concordance: Pathologists should receive specific training for HER2-low and ultralow scoring, for example, utilizing representative images. A local system for interobserver variability checks should be established, especially for HER2-low and ultralow tumors, which can be more subtle than HER2-positive cases.

Communication between medical oncologists and pathologists: Effective communication is crucial for assigning HER2 classes for T-DXd treatment. Although pathologists are now expected to distinguish IHC 0 (null) from IHC 0+ (ultralow), current evidence shows that this cannot always be achieved, and occasional misclassification is unavoidable. More than half of BCs that would previously have been classified simply as HER2 0 are HER2-ultralow. However, only a modest proportion of patients are eligible for T-DXd because eligibility is currently restricted to the metastatic setting and also relies on additional clinical criteria (eg, prior therapies and performance status). If a patient is being considered for T-DXd, but the existing report simply lists the tumor as HER2-negative, discussion regarding reevaluation of HER2 status, for example, reassessment or restaining of the original block or testing additional tissue blocks or specimens, as appropriate, is essential.

Recommendations:

1. Pathologists should be trained in HER2-low/ultralow scoring, with regular concordance checks to ensure accuracy.
2. Liaise with oncologists for potential reevaluation of cases originally classified as HER2-negative; if patients are eligible for ADC therapy, as determined by the oncologist.
3. Consider repeat HER2 testing in progressive or residual disease, given the known variability and dynamic changes in HER2 expression. The CAP synoptic templates are recommended for consistency across reports.

Other Points for Consideration

HER2 Immunohistochemistry Scores and Clinical Terminology

It is now apparent that the umbrella label "HER2-negative" needs to be further qualified for patients to be clinically identified as HER2-null, HER2-ultralow, or HER2-low, for potential T-DXd therapy in the metastatic BC setting. The 2 patterns described in the current ASCO/CAP guidelines for an IHC 0 score were also used in DB-06 to define the ultralow clinical category, but the distinction between $\leq$10% faint/barely perceptible, incomplete, membrane staining (ultralow/0+), and no staining at all (null/0) is subtle, and multiple studies have documented relatively poor interobserver agreement when pathologists subclassify HER2-negative BCs.[69,71,92,103]

In DB-06, 64% of tumors initially reported as IHC 0 were reclassified on central review as either HER2-low (24%) or HER2-ultralow (40%).[33] However, local laboratories had not been asked to separate IHC 0 into "null" and "ultralow," underscoring how evolving definitions can alter patient eligibility for ADCs.

We acknowledge concerns about the use of pharmaceutical-branded terminology to drive histopathological categorization of biomarkers and the risks of future drug-driven reclassification. It remains essential to base HER2 assessment on a single, standardized system, with current preference being the ASCO/CAP IHC criteria, to ensure consistency and avoid confusion. However, the terms "HER2-low" and "HER2-ultralow" have become widely adopted in clinical oncology and accurately reflect real-world communication needs. One practical approach is to report the IHC score according to ASCO/CAP guidelines while optionally including the corresponding clinical category in parentheses or commentary. This facilitates treatment planning without undermining diagnostic standards.

Recommendations:

A practical approach is to report the IHC score according to ASCO/CAP guidelines and, optionally, include the corresponding clinical category in parentheses or commentary. As per Table 1, HER2 IHC reporting generates 1 of the 5 current IHC categories (positive, equivocal, low, ultralow, or null). Once the reflex ISH results are available for those borderline/equivocal score 2+

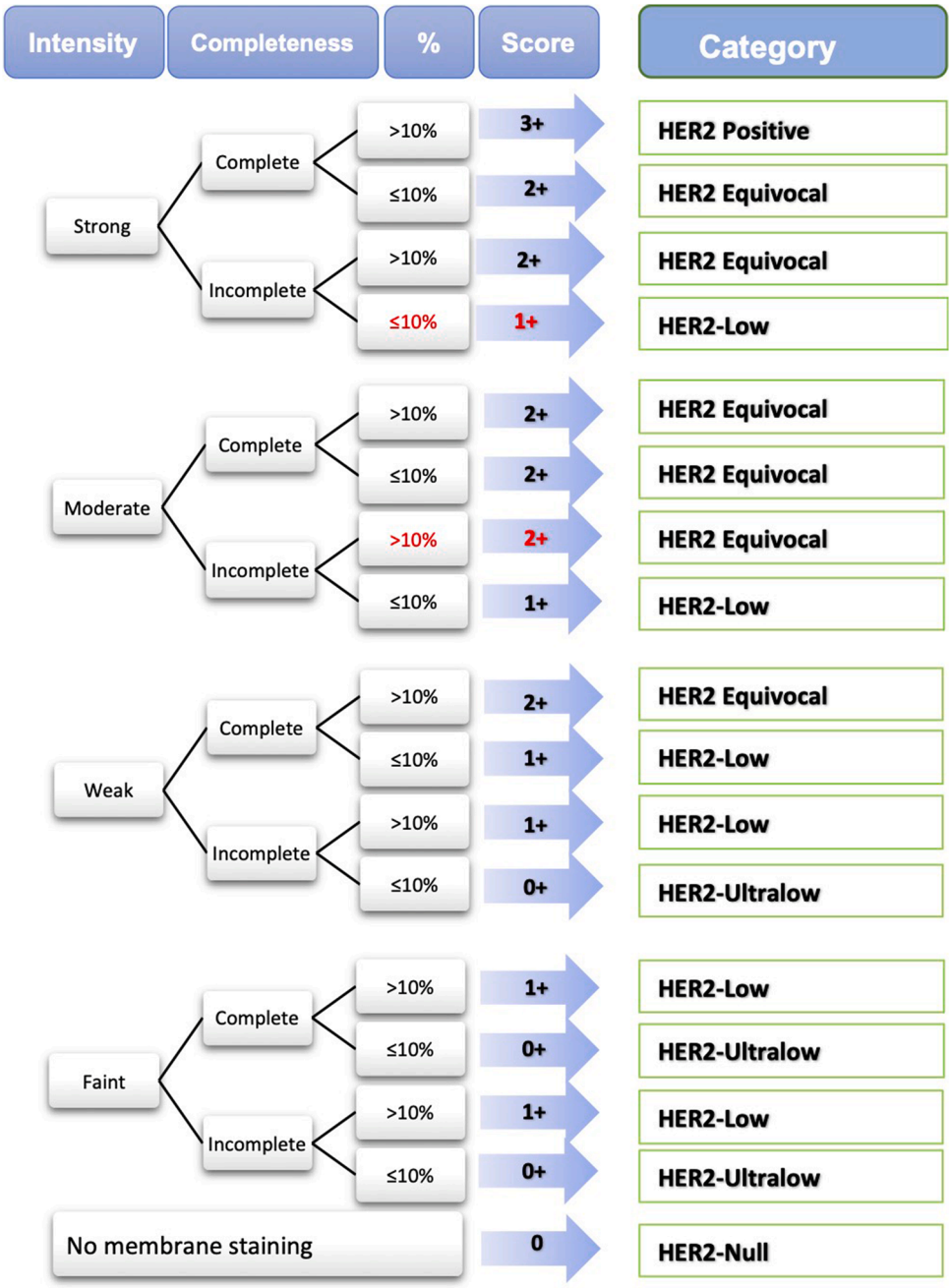

Figure 2.
Proposal for a comprehensive HER2 immunohistochemistry scoring system (modified from Rakha et al[77]).

cases, each tumor may be placed into 1 of the 4 final categories (positive, low, ultralow, or null).

HER2 Digital Pathology and Artificial Intelligence

AI algorithms are increasingly being explored to enhance the accuracy and reproducibility of HER2 IHC scoring, particularly in the assessment of HER2-low and ultralow BC. AI-based image analysis can offer a more objective and standardized approach by reducing interobserver variability and minimizing subjectivity in the interpretation of ambiguous cases, in particular, quantifying the percentage of tumor cells stained, staining intensity, and completeness of membrane staining.[104] However, one of the major limitations of these algorithms is the establishment of a reliable ground truth, and in tumors with HER2 scores 0+ and 1+, this is particularly challenging due to the inherent high degree of interobserver variability in these categories. A recent meta-analysis evaluating AI algorithms for HER2 scoring[104] demonstrated that performance improves with increasing HER2 scores. The highest pooled sensitivity was observed for HER2 IHC 3+ (0.97) with a specificity of 0.99, followed by HER2 2+ with a sensitivity of 0.89 and specificity of 0.96. For HER2 1+, the pooled sensitivity was lower (0.69; 95% CI, 0.57-0.79), with a specificity

of 0.94 and an area under the curve of 0.92.[105] Similarly, when the concordance across several AI algorithms for HER2 IHC scoring was evaluated,[106] the most significant discordance occurred in the intermediate categories (1+ and 2+), with higher agreement for scores 0 and 3+. Notably, the pattern of agreement among AI models across the HER2 score spectrum mirrors the agreement levels previously reported among pathologists.[106]

AI algorithms can only support the objectivity and consistency of HER2 scoring; they do not overcome other critical limitations inherent to the IHC process, as outlined above, including fixation-related issues, preanalytical variability, and tumor heterogeneity. Pathologist oversight remains essential to exclude nonrepresentative regions, particularly in situ lesions, which may exhibit higher HER2 expression than the invasive component. Although newer AI pipelines help mitigate some of these challenges by using myoepithelial immunostains to automatically exclude in situ carcinoma and by applying stain-normalization algorithms to reduce the impact of preanalytical variation and intratumoural heterogeneity, additional immunostains incur extra costs.

The clinical applicability and reliability of AI tools also depend on rigorous validation.[106] These scoring algorithms must be tested in well-characterized cohorts that include an adequate representation of HER2-low and ultralow tumors, rather than being trained and validated solely on data sets that dichotomize HER2 as positive or negative or relying on traditional 0 to 3+ scoring scales without a specific focus on the lower expression spectrum. Without such targeted validation, the clinical utility of AI in this emerging clinical context remains limited. Implementation also presupposes access to a digital pathology infrastructure, which is lacking in many laboratories worldwide. Furthermore, commercial licenses for these algorithms can be costly, raising cost-benefit concerns, especially in regions with constrained health care budgets. In laboratories without the requisite infrastructure or funding, adoption is likely to take years, which substantially limits the present-day utility of AI for routine HER2 assessment. At present, any results must still be reviewed by a pathologist.

Recommendations:

1. AI as a supportive tool: Validated AI tools should be utilized, when available, to assist with HER2 scoring. It is essential to maintain pathologist oversight alongside rigorous quality control and validation processes. Current evidence indicates that variability across different AI platforms is comparable to interobserver variability among pathologists. At present, AI, if used, should serve as an adjunct to, rather than a replacement for, expert pathological assessment.

The accurate reporting of HER2 status, particularly for HER2-low and ultralow BC, is vital for optimizing patient management and outcomes, but poses difficulties for laboratories, pathologists, and medical oncologists. Adherence to updated national and international HER2 guidelines, along with robust QA practices, ensures standardized testing and reporting, facilitating the appropriate use of targeted therapies. These guidelines will continue to evolve, incorporating further research, new insights, and technologies in the ongoing efforts to improve BC patient care.

Author Contributions

E.R. drafted the manuscript. All authors reviewed and commented on the draft and approved the final version.

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References

1. Harbeck N, Gnant M. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet*. 2017;389:1134–1150. https://doi.org/10.1016/S0140-6736(16)31891-8
2. Appert-Collin A, Hubert P, Crémel G, Bennasroune A. Role of ErbB receptors in cancer cell migration and invasion. *Front Pharmacol*. 2015;6:283. https://doi.org/10.3389/fphar.2015.00283
3. Ali R, Wendt MK. The paradoxical functions of EGFR during breast cancer progression. *Signal Transduct Target Ther*. 2017;2:1–7. https://doi.org/10.1038/sigtrans.2016.42
4. Konecny G, Pauletti G, Pegram M, et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. *J Natl Cancer Inst*. 2003;95:142–153. https://doi.org/10.1093/jnci/95.2.142
5. Sauter G, Lee J, Bartlett JM, Slamon DJ, Press MF. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. *J Clin Oncol*. 2009;27:1323–1333. https://doi.org/10.1200/JCO.2007.14.8197
6. Kallioniemi OP, Kallioniemi A, Kurisu W, et al. ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. *Proc Natl Acad Sci USA*. 1992;89:5321–5325. https://doi.org/10.1073/pnas.89.12.5321
7. Marchiò C, Annaratone L, Marques A, et al. Evolving concepts in HER2 evaluation in breast cancer: Heterogeneity, HER2-low carcinomas and beyond. *Semin Cancer Biol*. 2021;72:123–135. https://doi.org/10.1016/j.semcancer.2020.02.016
8. Bradley R, Braybrooke J, Gray R, et al. Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. *Lancet Oncol*. 2021;22:1139–1150. https://doi.org/10.1016/S1470-2045(21)00288-6
9. 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. https://doi.org/10.3389/fmolb.2023.1176309
10. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001;344:783–792. https://doi.org/10.1056/NEJM200103153441101
11. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005;353:1673–1684. https://doi.org/10.1056/NEJMoa052122
12. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*. 2007;131:18–43. https://doi.org/10.5858/2007-131-18-ASOCCO
13. Ellis IO, Dowsett M, Bartlett J, et al. Recommendations for HER2 testing in the UK. *J Clin Pathol*. 2000;53:890–892. https://doi.org/10.1136/jcp.53.12.890

14. Rakha EA, Pinder SE, Bartlett JM, et al. Updated UK Recommendations for HER2 assessment in breast cancer. *J Clin Pathol*. 2015;68:93–99. https://doi.org/10.1136/jclinpath-2014-202571
15. Carlson RW, Moench SJ, Hammond ME, et al. HER2 testing in breast cancer: NCCN Task Force report and recommendations. *J Natl Compr Canc Netw*. 2006;4(Suppl 3):S1–S22;quiz S23-S24. https://doi.org/10.6004/jnccn.2006.2003
16. Lambein K, Praet M, Forsyth R, et al. Relationship between pathological features, HER2 protein expression and HER2 and CEP17 copy number in breast cancer: biological and methodological considerations. *J Clin Pathol*. 2011;64:200–207. https://doi.org/10.1136/jcp.2010.084863
17. Dowsett M, Procter M, McCaskill-Stevens W, et al. Disease-free survival according to degree of HER2 amplification for patients treated with adjuvant chemotherapy with or without 1 year of trastuzumab: the HERA Trial. *J Clin Oncol*. 2009;27:2962–2969. https://doi.org/10.1200/JCO.2008.19.7939
18. Valero V, Forbes J, Pegram MD, et al. Multicenter phase III randomized trial comparing docetaxel and trastuzumab with docetaxel, carboplatin, and trastuzumab as first-line chemotherapy for patients with HER2-gene-amplified metastatic breast cancer (BCIRG 007 study): two highly active therapeutic regimens. *J Clin Oncol*. 2011;29:149–156. https://doi.org/10.1200/JCO.2010.28.6450
19. 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;38:444. https://doi.org/10.1200/JCO.19.01455
20. Subbiah IM, Gonzalez-Angulo AM. Advances and future directions in the targeting of HER2-positive breast cancer: implications for the future. *Curr Treat Options Oncol*. 2014;15:41–54. https://doi.org/10.1007/s11864-013-0262-4
21. Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov*. 2023;22:101–126. https://doi.org/10.1038/s41573-022-00579-0
22. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*. 2005;353:1659–1672. https://doi.org/10.1056/NEJMoa052306
23. Gianni L, Pienkowski T, Im YH, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *Lancet Oncol*. 2016;17:791–800. https://doi.org/10.1016/S1470-2045(16)00163-7
24. Dodson A, Parry S, Ibrahim M, et al. Breast cancer biomarkers in clinical testing: analysis of a UK national external quality assessment scheme for immunocytochemistry and in situ hybridisation database containing results from 199 300 patients. *J Pathol Clin Res*. 2018;4:262–273. https://doi.org/10.1002/cjp2.112
25. Nielsen S, Bzorek M, Vyberg M, Roge R. Lessons learned, challenges taken, and actions made for "precision" immunohistochemistry. Analysis and perspectives from the NordiQC proficiency testing program. *Appl Immunohistochem Mol Morphol*. 2023;31:452–458. https://doi.org/10.1097/PAI.0000000000001071
26. 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;41:3867–3872. https://doi.org/10.1200/JCO.22.02864
27. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*. 2013;31:3997–4013. https://doi.org/10.1200/JCO.2013.50.9984
28. 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;36:2105–2122. https://doi.org/10.1200/JCO.2018.77.8738
29. Ellis IO, Bartlett J, Dowsett M, et al. Best Practice No 176: updated recommendations for HER2 testing in the UK. *J Clin Pathol*. 2004;57:233–237. https://doi.org/10.1136/jcp.2003.007724
30. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007;25:118–145. https://doi.org/10.1200/JCO.2006.09.2775
31. Lambein K, Van Bockstal M, Denys H, Libbrecht L. 2013 update of the American Society of Clinical Oncology/College of American Pathologists guideline for human epidermal growth factor receptor 2 testing: impact on immunohistochemistry-negative breast cancers. *J Clin Oncol*. 2014;32:1856–1857. https://doi.org/10.1200/JCO.2013.54.2530
32. Modi S, Jacot W, Yamashita T, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med*. 2022;387:9–20. https://doi.org/10.1056/NEJMoa2203690
33. Bardia A, Hu X, Dent R, et al. Trastuzumab deruxtecan after endocrine therapy in metastatic breast cancer. *N Engl J Med*. 2024;391:2110–2122. https://doi.org/10.1056/NEJMoa2407086
34. Rinnerthaler G, Gampenrieder SP, Greil R. HER2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer. *Int J Mol Sci*. 2019;20:1115. https://doi.org/10.3390/ijms20051115
35. Tarantino P, Hamilton E, Tolaney SM, et al. HER2-low breast cancer: pathological and clinical landscape. *J Clin Oncol*. 2020;38:1951–1962. https://doi.org/10.1200/JCO.19.02488
36. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Version 4.2025. 2025. Accessed May 15, 2025. https://www.nccn.org/guidelines/guidelines-detail?id=1419
37. European Society for Medical Oncology. ESMO Expert Consensus Statements on the Definition, Diagnosis, and Management of HER2-Low Breast Cancer. 2023. Accessed May 15, 2025. https://www.esmo.org/guidelines/esmo-expert-consensus-statements/esmo-expert-consensus-statements-on-the-definition-diagnosis-and-management-of-her2-low-breast-cancer
38. Miglietta F, Griguolo G, Bottosso M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer*. 2021;7:137. https://doi.org/10.1038/s41523-021-00343-4
39. 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;190:155–163. https://doi.org/10.1007/s10549-021-06365-7
40. 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;35:1075–1082. https://doi.org/10.1016/j.semcancer.2020.02.016
41. van den Ende NS, Smid M, Timmermans A, et al. HER2-low breast cancer shows a lower immune response compared to HER2-negative cases. *Sci Rep*. 2022;12:12974. https://doi.org/10.1038/s41598-022-16898-6
42. 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;22:1151–1161. https://doi.org/10.1016/S1470-2045(21)00301-6
43. Molinelli C, Jacobs F, Agostinetto E, et al. Prognostic value of HER2-low status in breast cancer: a systematic review and meta-analysis. *ESMO Open*. 2023;8:101592. https://doi.org/10.1016/j.esmoop.2023.101592
44. Agostinetto E, Rediti M, Fimereli D, et al. HER2-low breast cancer: molecular characteristics and prognosis. *Cancers*. 2021;13:2824. https://doi.org/10.3390/cancers13112824
45. 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(2):234–241. https://doi.org/10.1007/s12282-021-01303-3
46. 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*. 2021;13:6059. https://doi.org/10.3390/cancers13236059
47. 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;7:1. https://doi.org/10.1038/s41523-020-00208-2
48. Baez-Navarro X, van Bockstal MR, Andrinopoulou ER, van Deurzen CHM. HER2-low breast cancer: incidence, clinicopathologic features, and survival outcomes from real-world data of a large nationwide cohort. *Mod Pathol*. 2023;36:100087. https://doi.org/10.1016/j.modpat.2022.100087
49. Berrino E, Annaratone L, Bellomo SE, et al. Integrative genomic and transcriptomic analyses illuminate the ontology of HER2-low breast carcinomas. *Genome Med*. 2022;14:98. https://doi.org/10.1186/s13073-022-01104-z
50. Lai H-Z, Han J-R, Fu X, et al. Targeted approaches to HER2-low breast cancer: current practice and future directions. *Cancers*. 2022;14:3774. https://doi.org/10.3390/cancers14153774
51. Molinelli C, Jacobs F, Marchiò C, et al. HER2-low breast cancer: where are we? *Breast Care*. 2022;17:533–545. https://doi.org/10.1159/000527391
52. Nicolò E, Tarantino P, Curigliano G. Biology and treatment of HER2-low breast cancer. *Hematol Oncol Clin*. 2023;37:117–132. https://doi.org/10.1016/j.hoc.2022.08.013
53. Atallah NM, Haque M, Quinn C, et al. Characterisation of luminal and triple-negative breast cancer with HER2 Low protein expression. *Eur J Cancer*. 2023;195:113371. https://doi.org/10.1016/j.ejca.2023.113371
54. Tarantino P, Morganti S, Curigliano G. Biologic therapy for advanced breast cancer: recent advances and future directions. *Expert Opin Biol Ther*. 2020;20:1009–1024. https://doi.org/10.1080/14712598.2020.1752176
55. Ivanova M, Porta FM, D'Ercole M, et al. Standardized pathology report for HER2 testing in compliance with 2023 ASCO/CAP updates and 2023 ESMO consensus statements on HER2-low breast cancer. *Virchows Archiv*. 2024;484:3–14. https://doi.org/10.1007/s00428-023-03656-w
56. 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;107:1039–1046. https://doi.org/10.1111/cas.12966

57. Tsao LC, Wang JS, Ma X, et al. Effective extracellular payload release and immunomodulatory interactions govern the therapeutic effect of trastuzumab deruxtecan (T-DXd). *Nat Commun*. 2025;16:3167. https://doi.org/10.1038/s41467-025-58266-8
58. Saura C, Modi S, Krop I, et al. Trastuzumab deruxtecan in previously treated patients with HER2-positive metastatic breast cancer: updated survival results from a phase II trial (DESTINY-Breast01). *Ann Oncol*. 2024;35:302–307. https://doi.org/10.1016/j.annonc.2023.12.001
59. Cortes J, Kim SB, Chung WP, et al. Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. *N Engl J Med*. 2022;386:1143–1154. https://doi.org/10.1056/NEJMoa2115022
60. Mosele F, Deluche E, Lusque A, et al. Trastuzumab deruxtecan in metastatic breast cancer with variable HER2 expression: the phase 2 DAISY trial. *Nat Med*. 2023;29:2110–2120. https://doi.org/10.1038/s41591-023-02478-2
61. FDA approves fam-trastuzumab deruxtecan-nxki for unresectable or metastatic HR-positive. HER2-low or HER2-ultralow breast cancer. 2025. Accessed June 10, 2025. https://www.fda.gov
62. Tarantino P, Viale G, Press MF, et al. ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer. *Ann Oncol*. 2023;34:645–659. https://doi.org/10.1016/j.annonc.2023.05.008
63. College of American Pathologists. Reporting Template for Reporting Results of Biomarker Testing of Specimens from Patients With Carcinoma of the Breast. Northfield, IL: College of American Pathologists. 2025. Accessed January 9, 2025. https://documents.cap.org/protocols/cp-breast-biomarker-22-rf.pdf
64. Karakas C, Tyburski H, Turner BM, et al. Interobserver and interantibody reproducibility of HER2 immunohistochemical scoring in an enriched HER2-low-expressing breast cancer cohort. *Am J Clin Pathol*. 2023;159:484–491. https://doi.org/10.1093/ajcp/aqac184
65. Robbins CJ, Fernandez AI, Han G, et al. Multi-institutional assessment of pathologist scoring HER2 immunohistochemistry. *Mod Pathol*. 2023;36:100032. https://doi.org/10.1016/j.modpat.2022.100032
66. Zaakouk M, Quinn C, Provenzano E, et al. Concordance of HER2-low scoring in breast carcinoma among expert pathologists in the United Kingdom and the republic of Ireland -on behalf of the UK national coordinating committee for breast pathology. *Breast*. 2023;70:82–91. https://doi.org/10.1016/j.breast.2023.06.005
67. Fernandez AI, Liu M, Bellizzi A, et al. Examination of low ERBB2 protein expression in breast cancer tissue. *JAMA Oncol*. 2022;8:1–4. https://doi.org/10.1001/jamaoncol.2021.7239
68. Tarantino P, Curigliano G, Tolaney SM. Navigating the HER2-low paradigm in breast oncology: new standards, future horizons. *Cancer Discov*. 2022;12:2026–2030. https://doi.org/10.1158/2159-8290.CD-22-0703
69. Turashvili G, Gao Y, Ai DA, et al. Low interobserver agreement among subspecialised breast pathologists in evaluating HER2-low breast cancer. *J Clin Pathol*. 2024;77:815–821. https://doi.org/10.1136/jcp-2023-209055
70. Baez-Navarro X, van Bockstal MR, Nawawi D, et al. Interobserver variation in the assessment of immunohistochemistry expression levels in HER2-negative breast cancer: can we improve the identification of low levels of HER2 expression by adjusting the criteria? An international interobserver study. *Mod Pathol*. 2023;36:100009. https://doi.org/10.1016/j.modpat.2022.100009
71. Wu S, Shang J, Li Z, et al. Interobserver consistency and diagnostic challenges in HER2-ultralow breast cancer: a multicenter study. *ESMO Open*. 2025;10:104127. https://doi.org/10.1016/j.esmoop.2024.104127
72. Venetis K, Crimini E, Sajjadi E, et al. HER2 low, ultra-low, and novel complementary biomarkers: expanding the spectrum of HER2 positivity in breast cancer. *Front Mol Biosci*. 2022;9:834651. https://doi.org/10.3389/fmolb.2022.834651
73. Bar Y, Dedeoglu AS, Fell GG, et al. Dynamic HER2-low status among patients with triple negative breast cancer (TNBC): the impact of repeat biopsies. *J Clin Oncol*. 2023;41:1005-1005 https://doi.org/10.1200/JCO.2023.41.16_suppl.1005
74. Zhao Y, Chen X, Wang Y, et al. Stable or at least once HER2-low status during neoadjuvant chemotherapy confers survival benefit in patients with breast cancer. *Ann Med*. 2024;56:2409343. https://doi.org/10.1080/07853890.2024.2409343
75. Denkert C, Martín M, Untch M, et al. Outcome analysis of HER2-zero or HER2-low hormone receptor-positive (HR+) breast cancer patients: characterization of the molecular phenotype in combination with molecular subtyping. *Cancer Res*. 2023;83(5 Suppl):HER2-06 https://doi.org/10.1158/1538-7445.SABCS22-HER2-06
76. Nicolò E, Boscolo Bielo L, Curigliano G, Tarantino P. The HER2-low revolution in breast oncology: steps forward and emerging challenges. *Ther Adv Med Oncol*. 2023;15:17588359231152842. https://doi.org/10.1177/17588359231152842
77. Rakha EA, Tan PH, Quinn C, et al. UK recommendations for HER2 assessment in breast cancer: an update. *J Clin Pathol*. 2023;76:217–227. https://doi.org/10.1136/jcp-2022-208632
78. Breast Cancer Expert Committee of National Quality Control Center for Cancer. Breast Cancer Expert Committee of China Anti-Cancer Association; Cancer Drug Clinical Research Committee of China Anti-Cancer Association. Guidelines for diagnosis and treatment of advanced breast cancer in China (2022 edition). *J Natl Cancer Cent*. 2024;4:107–127. https://doi.org/10.1016/j.jncc.2023.12.001
79. Tozbikian G, Bui MM, Hicks DG, et al. Best practices for achieving consensus in HER2-low expression in breast cancer: current perspectives from practising pathologists. *Histopathology*. 2024;85:489–502. https://doi.org/10.1111/his.15275
80. Yildiz-Aktas IZ, Dabbs DJ, Cooper KL, et al. The effect of 96-hour formalin fixation on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Am J Clin Pathol*. 2012;137:691–698. https://doi.org/10.1309/AJCPQRAG67GJRPMT
81. Tong LC, Nelson N, Tsourigiannis J, Mulligan AM. The effect of prolonged fixation on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast cancer: a prospective study. *Am J Surg Pathol*. 2011;35:545–552. https://doi.org/10.1097/PAS.0b013e31820e6237
82. Maleki S, Dorokhova O, Sunkara J, et al. Estrogen, progesterone, and HER-2 receptor immunostaining in cytology: the effect of varied fixation on human breast cancer cells. *Diagn Cytopathol*. 2013;41:864–870. https://doi.org/10.1002/dc.22973
83. 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. https://doi.org/10.3390/cancers14246200
84. Lin M, Luo T, Jin Y, et al. HER2-low heterogeneity between primary and paired recurrent/metastatic breast cancer: implications in treatment and prognosis. *Cancer*. 2024;130:851–862. https://doi.org/10.1002/cncr.35101
85. Yang E, D'Alfonso TM, Morrow M, Brogi E, Wen HY. Discordance of human epidermal growth factor receptor 2-low status between breast primary and distant metastases with clinical-pathological correlation. *Histopathology*. 2025;86:441–449. https://doi.org/10.1111/his.15346
86. Prat A, Modi S, Tsurutani J, Cameron N, Garrido HC. Abstract, HER2-18 determination of HER2-low status in tumors of patients with unresectable and/or metastatic breast cancer in DESTINY-Breast04. San Antonio Breast Cancer Symposium. 2022.
87. Hempenius MA, Eenkhoorn MA, Hoeg H, et al. Quantitative comparison of immunohistochemical HER2-low detection in an interlaboratory study. *Histopathology*. 2024;85:920–928. https://doi.org/10.1111/his.15273
88. Parry S, Zabaglo L, Wilkinson D, Dodson A. The UK National External Quality Assessment Scheme for Immunocytochemistry and In-Situ Hybridisation (UK NEQAS ICC & ISH) HER2-low EQA programme: a review of results and factors influencing successful staining. *Diagn Histopathol*. 2025;31:131–144. https://doi.org/10.1016/j.mpdhp.2024.12.001
89. Larson JS, Goodman LJ, Tan Y, et al. Analytical validation of a highly quantitative, sensitive, accurate, and reproducible assay (HERmark®) for the measurement of HER2 total protein and HER2 homodimers in FFPE breast cancer tumor specimens. *Pathol Res Int*. 2010;2010:814176. https://doi.org/10.4061/2010/814176
90. Yardley DA, Kaufman PA, Huang W, et al. Quantitative measurement of HER2 expression in breast cancers: comparison with 'real-world'routine HER2 testing in a multicenter Collaborative Biomarker Study and correlation with overall survival. *Breast Cancer Res*. 2015;17:1–13. https://doi.org/10.1186/s13058-015-0543-x
91. Harigopal M, Barlow WE, Tedeschi G, et al. Multiplexed assessment of the Southwest Oncology Group-directed Intergroup Breast Cancer Trial S9313 by AQUA shows that both high and low levels of HER2 are associated with poor outcome. *Am J Pathol*. 2010;176:1639–1647. https://doi.org/10.2353/ajpath.2010.090711
92. Moutafi M, Robbins CJ, Yaghoobi V, et al. Quantitative measurement of HER2 expression to subclassify ERBB2 unamplified breast cancer. *Lab Invest*. 2022;102:1101–1108. https://doi.org/10.1038/s41374-022-00804-9
93. Jensen K, Krusenstjerna-Hafstrøm R, Lohse J, Petersen KH, Derand H. A novel quantitative immunohistochemistry method for precise protein measurements directly in formalin-fixed, paraffin-embedded specimens: analytical performance measuring HER2. *Mod Pathol*. 2017;30:180–193. https://doi.org/10.1038/modpathol.2016.176
94. Griguolo G, Brasó-Maristany F, Gonzalez-Farre B, et al. ERBB2 mRNA expression and response to ado-trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. *Cancers*. 2020;12:1902. https://doi.org/10.3390/cancers12071902
95. Atallah N, Makhlouf S, Li X, et al. Prediction of response to anti-HER2 therapy using a multigene assay. *Mod Pathol*. 2025;38:100713. https://doi.org/10.1016/j.modpat.2025.100713
96. Li X, Lee JH, Gao Y, et al. Correlation of HER2 protein level with mRNA level quantified by RNAscope in breast cancer. *Mod Pathol*. 2024;37:100408. https://doi.org/10.1016/j.modpat.2023.100408
97. Atallah NM, Toss MS, Green AR, et al. Refining the definition of HER2-low class in invasive breast cancer. *Histopathology*. 2022;81:770–785. https://doi.org/10.1111/his.14780
98. Xiao Y, Gao X, Maragh S, Telford WG, Tona A. Cell lines as candidate reference materials for quality control of ERBB2 amplification and

expression assays in breast cancer. *Clin Chem*. 2009;55:1307–1315. https://doi.org/10.1373/clinchem.2008.120576
99. Leica Biosystems. Bond™ Oracle™ HER2 IHC System Interpretation Guide. Newcastle: Leica Biosystems; 2022. Accessed October 8, 2025. https://www.leicabiosystems.com/sites/default/files/2020-10/Oracle%20HER2%20Interpretation%20Guide%20%28Breast%29.pdf
100. Sompuram SR, Vani K, Schaedle AK, Balasubramanian A, Bogen SA. Quantitative assessment of immunohistochemistry laboratory performance by measuring analytic response curves and limits of detection. *Arch Pathol Lab Med*. 2018;142:851–862. https://doi.org/10.5858/arpa.2017-0330-OA
101. Wolff AC, Somerfield MR, Dowsett M, et al. Human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med*. 2023;147:993–1000. https://doi.org/10.5858/arpa.2023-0950-SA
102. Tan PH, Mihir G, Laokulrath N, Rakha E. Practical approach to scoring HER2 immunohistochemistry in breast cancer in the wake of updated guidelines. *Histopathology*. 2024;84:715–718. https://doi.org/10.1111/his.15117
103. Pauzi SHM, Masir N, Yahaya A, et al. HER2 testing by immunohistochemistry in breast cancer: a multicenter proficiency ring study. *Indian J Pathol Microbiol*. 2021;64:677–682. https://doi.org/10.4103/IJPM.IJPM_983_20
104. Wu S, Li X, Miao J, et al. Artificial intelligence for assisted HER2 immunohistochemistry evaluation of breast cancer: a systematic review and meta-analysis. *Pathol Res Pract*. 2024;260:155472. https://doi.org/10.1016/j.prp.2024.155472
105. Wrobel A, Vandenberghe M, Scott M, et al. Accuracy of human epidermal growth factor receptor 2 (HER2) immunohistochemistry scoring by pathologists in breast cancer, including the HER2-low cutoff : HER2 IHC scoring concordance in breast cancer. *Diagn Pathol*. 2025;20:35. https://doi.org/10.1186/s13000-025-01624-3
106. McKelvey B, Torres-Saavedra PA, Li J, et al. Agreement across 10 artificial intelligence models in assessing HER2 in breast cancer whole slide images: findings from the friends of cancer research digital PATH Project. 2024. Accessed October 8, 2025. https://friendsofcancerresearch.org/wp-content/uploads/SABCS-Poster_Digital-PATH-Project-Findings.pdf