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PERSPECTIVE

HER2 ultralow in metastatic breast cancer: Diagnostic boundaries in the era of antibody–drug conjugates

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Abstract

The introduction of antibody–drug conjugates (ADCs) such as trastuzumab deruxtecan (T-DXd) has challenged the traditional binary classification of HER2 in breast cancer. Evidence from the DESTINY-Breast04/06 and DAISY trials demonstrates clinically meaningful antitumour activity across a continuum of HER2 expression, including HER2-low and HER2-ultralow categories, while efficacy in HER2-null tumours remains uncertain. Despite this graded biological response, regulatory criteria remain categorical, requiring detectable membrane staining for treatment eligibility and excluding IHC 0 tumours. Pathologists face diagnostic challenges at the null–ultralow boundary, where conventional immunohistochemistry (IHC) assays operate near their analytical limits, reproducibility is constrained, and inter-observer variability is high. Pathologists should exercise caution when assigning IHC 0, and practical measures such as reflexive re-evaluation, re-staining of alternative blocks, and referral to high-sensitivity laboratories are recommended. Transparent communication of analytical uncertainty is essential to optimise patient access to ADC therapy.

Keywords: HER2-null, HER2-ultralow, Antibody–drug conjugates

INTRODUCTION

Historically, HER2 status in breast cancer has been interpreted through a binary lens: positive (IHC 3+ or IHC 2+/ISH-amplified) or negative. This framework effectively guides conventional anti-HER2 therapies, whose efficacies depend on high-level HER2 expression driving oncogenic addiction. The emergence of antibody–drug conjugates like trastuzumab deruxtecan (T-DXd) now challenges this long-standing binary classification. T-DXd operates through receptor-mediated internalization of a cytotoxic payload and a bystander killing effect. Unlike classical anti-HER2 agents, it requires substantially lower HER2 expression to be active.[1,2] Consequently, clinically meaningful responses have been observed across a broader expression continuum, including HER2-low (IHC 1+/2+ ISH−) and HER2-ultralow (IHC 0 with faint/incomplete membrane staining ≤10%, designated '0+' in the College of American Pathologists (CAP) reporting template) categories.[3–5]

While regulatory approvals restrict T-DXd to HER2-low or HER2-ultralow, exploratory data suggest activity in subsets of tumours classified as pure IHC 0.[6] This discrepancy calls for careful re-examination of our reliance on a single categorical "IHC 0" designation—particularly at a boundary where inherent analytical variability in current platforms can directly influence therapeutic eligibility.

Building on recent trial developments, this perspective synthesises clinical evidence for a graded response model, characterises the analytical challenges of low HER2 expression, evaluates standardised reporting frameworks, and proposes practical strategies for routine pathology practice.

The clinical case for a graded response model

Level 1 evidence from the DESTINY-Breast04 trial established T-DXd's efficacy in HER2-low metastatic breast cancer, demonstrating significant improvements in progression-free and overall survival versus chemotherapy.[3,4] The DESTINY-Breast06 trial subsequently confirmed activity in hormone receptor–positive disease across HER2-low and HER2-ultralow

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subgroups following endocrine therapy. Benefit was demonstrable in ultralow cases exhibiting faint membranous staining.[5]

The phase II DAISY trial evaluated T-DXd across the full HER2 expression spectrum and provided direct evidence of a graded response pattern.[6] Objective response rates were 70.6% (95% CI 58.3–81.0) in HER2-overexpressing tumours (IHC 3+), 37.5% (95% CI 26.4–49.7) in HER2-low disease (IHC 1+ or IHC 2+/ISH-negative), and 29.7% (95% CI 15.9–47.0) in tumours historically classified as HER2 IHC 0. Importantly, the IHC 0 cohort encompassed both HER2-null and HER2-ultralow tumours. It demonstrated substantial analytical instability: central re-evaluation identified previously unrecognised HER2 expression in nearly half of cases initially scored as IHC 0 on baseline biopsy. Exploratory analyses documented objective responses in tumours with detectable expression on re-evaluation (HER2-ultralow/low: 40%, 95% CI 16.3–67.7) and in persistently undetectable cases (HER2-null: 25%, 95% CI 7.3–52.4). The latter subgroup was small, however, and confidence intervals were wide.

Real-world cohorts, including patients with brain metastases, report systemic response rates of approximately 33% in HER2-low/zero groups, with occasional responses in IHC 0 intracranial lesions.[7] These series are, however, retrospective, limited in size, and subject to selection bias.

Together, the available evidence favours a probabilistic model, where the likelihood of response increases with HER2 expression levels. Regulatory eligibility and clinical decision-making, however, remain contingent upon defined low and ultralow categories requiring detectable staining. This mismatch forms the core diagnostic challenge: a clinical continuum of graded response collides with categorical regulatory criteria.

Analytical challenges at low HER2 expression levels

Traditional HER2 IHC assays were developed and optimised to detect high-level overexpression (IHC 3+), emphasising specificity and positive predictive value for patient selection for trastuzumab-based therapies. Repurposing these platforms to distinguish HER2-low and HER2-ultralow from null expression requires substantially greater analytical sensitivity and negative predictive value—performance characteristics that differ fundamentally from those for which these assays were designed and validated.

Assay Limitations at Low Expression Levels

Current standard HER2 IHC assays (e.g., Roche/Ventana 4B5) have limits of detection in the range of 30,000–60,000 HER2 molecules per cell. In the CASI-01 study, these assays performed well for identifying HER2 overexpression (IHC 3+): sensitivity 85.7% (95% CI 63.7–97.0%) and specificity 100% (95% CI 92.8–100%) against ISH-confirmed amplification.[8] However, they exhibited poor dynamic range for discriminating low HER2 scores. Only higher-sensitivity assays combined with digital image analysis achieved proportional discrimination in the low-expression range, with a reported six-fold improvement in dynamic range.[8]

At the lower end of the expression spectrum, this analytical constraint carries important clinical implications. As HER2 staining nears the assay's analytical detection limit, minor variations in intensity often fail to produce consistent categorical assignments among pathologists. As a result, distinctions among HER2-null, ultralow, and low expression categories may partly reflect variability in assay performance and interpretive readout rather than precise quantitative differences in HER2 protein levels.

Inter-Observer Variability at the Null-Ultralow Boundary

Identification of HER2-ultralow expression requires detection of faint, incomplete membranous staining that frequently overlaps with the assay's analytical noise floor. Because the transition from a HER2-null (completely negative) result to a faint ultralow result lacks a clear morphological boundary, pathologists must rely on a subjective perceptibility threshold that resists standardisation.

The difficulty of this classification was highlighted by a 2025 multicentre study[9] involving 36 pathologists. When tasked with subdividing IHC 0 specimens into HER2-null, HER2-ultralow, and HER2-low categories, the pathologists achieved low interobserver consistency (Fleiss $\kappa = 0.230$), reaching high agreement in only 4% of cases. However, when these cases were grouped together simply as IHC 0 without sub-classification as HER2-null or HER2-ultralow, high agreement rose significantly

to 32%. Furthermore, while the group reached a consensus that matched historical records in 72% of cases overall, this accuracy dropped to 54% when they attempted to subdivide the results into null and ultralow. The central–local discordance rate of 41.2% observed in DAISY further illustrates the magnitude of this variability in practice.[6]

Additional Biological Sources of Variability

HER2 expression is subject to spatial intra-tumoural heterogeneity and temporal evolution under treatment selection pressure. As a result, HER2 status at the primary site may not reflect that of metastatic lesions sampled later.[10] These sources of biological variability are independent of and additive to the technical constraints of current IHC platforms. Together, they define the practical ceiling of what categorical IHC reporting can achieve at the low-expression boundary under existing analytical conditions.

The College of American Pathologists (CAP) standardised comment: Scope and limitations

Following recent trial developments, the CAP reporting template now includes an optional standardised comment for HER2 IHC 0, 0+, 1+, or 2+ results.[11] Its aim is to harmonise diagnostic language with the therapeutic categories used in pivotal trials. The recommended text states:

"In the DESTINY-Breast 04 and 06 trials, 'HER2 low' was considered IHC Score 1+ or 2+/ISH negative, and 'HER2 ultralow' was HER2 IHC Score of 0 (pattern 0+) with membrane staining that is incomplete and faint/barely perceptible in less than or equal to 10% of tumour cells. Breast cancers with these staining patterns may be eligible for treatment with trastuzumab-deruxtecan in the metastatic setting (but those with no staining, IHC 0, are currently excluded)."

(Note: "0+" denotes the CAP template's shorthand for the ultralow staining pattern within the IHC 0 category, consistent with the ASCO/CAP definition of HER2-ultralow.)

The practical value of this comment lies in its preservation of categorical IHC scores required for regulatory and reimbursement purposes, while providing oncologists with clinical context derived from pivotal trials. However, as a reporting tool rather than an analytical intervention, the template has important limitations. Notably, the template neither acknowledges the documented interobserver variability at this threshold nor evaluates whether current assays can reliably distinguish IHC 0 from IHC 0+. It also offers no guidance for cases at the immediate limit of assay detectability. In practice, the comment provides useful clinical context for the categorical score but stops short of addressing the underlying analytical uncertainty. Pathologists adopting this template should recognise it as a communication aid that facilitates multidisciplinary dialogue rather than a solution to the analytical problem it describes.

The technical–ethical framework: Probabilistic reality versus categorical requirement

Diagnostic pathology is premised on the delivery of definitive categorical results to support clinical decision-making. The requirement to report a binary "IHC 0" result at the limit of assay detection introduces a tension between this expectation and the limited analytical resolution of the assay at this boundary.

In a representative clinical scenario, a patient with heavily pretreated metastatic breast cancer whose biopsy is interpreted as IHC 0 is rendered ineligible for T-DXd. If subsequent central review identifies ultralow staining, the initial result need not reflect diagnostic error—it may simply reflect the inherent variability of measurements near the assay's sensitivity limits.

The DAISY trial data further complicates this picture. Approximately 25% of patients with undetectable HER2 expression derived clinical benefit from T-DXd, suggesting that antitumour activity can occur through mechanisms not captured by current IHC thresholds.[6] This implies that even analytically perfect null-ultralow discrimination would not fully predict response probability—the biological signal itself is probabilistic.

Addressing this challenge requires transparent acknowledgment of analytical limitations within multidisciplinary teams and at tumour boards. Pathologists should convey the inherent uncertainty in low-expression classifications rather than presenting categorical results as fully definitive.

Conclusion: Embracing analytical honesty in HER2 reporting

Navigating HER2 testing in the ADC era requires candid acknowledgment of IHC's inherent limitations, particularly at the HER2-null-ultralow boundary. While the universal

adoption of the CAP optional standardised comment preserves categorical scores for regulatory purposes, an IHC 0 assignment must be approached with caution. Given the limited reproducibility near the null-ultralow threshold, pathologists should initiate a reflexive re-evaluation when initial samples are suboptimal, metastatic site material differs from the primary, or clinical suspicion is high due to prior HER2 heterogeneity or exhaustion of standard treatment options.

Prioritising the re-staining of alternative blocks or referral to centres with high-sensitivity assays can mitigate the impact of analytical variability. In resource-constrained settings, rigorous peer review and targeted training in ultralow pattern recognition offer immediate, feasible solutions. Oncologists must understand that an IHC 0 result represents a below-threshold finding under specific analytical conditions, not a confirmed biological state. These proposals align with and extend recent national recommendations from the Malaysian Breast Pathology Working Group.[12] Until next-generation assays with validated low-expression performance become widely available, transparent communication of this uncertainty is more than good practice. It is an obligation to patients whose treatment eligibility hinges on this borderline distinction.

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REFERENCES

1. 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.
2. 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(7):1039–1046.
3. Modi S, Jacot W, Yamashita T, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med. 2022;387(1):9-20.
4. Modi S, Jacot W, Iwata H, et al. Trastuzumab deruxtecan in HER2-low metastatic breast cancer: long-term survival analysis of the randomized, phase 3 DESTINY-Breast04 trial. Nat Med. 2025;31(12):4205-4213
5. Bardia A, Hu X, Dent R, et al. Trastuzumab Deruxtecan after Endocrine Therapy in Metastatic Breast Cancer. N Engl J Med. 2024;391(22):2110-2122..
6. 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(8):2110-2120.
7. Zhou Q, Li Y, He N, et al. Efficacy and Safety of T-DXd in HER2+ and Low/Zero Metastatic BCBM: A Retrospective Multicenter Real-World Study. *Cancer Sci*. 2025;116(11):3149-3159.
8. Dabbs DJ, Torlakovic E, Nielsen S, et al. New standards in HER2-low testing: the CASI-01 comparative methods study. EBioMedicine. 2025;120:105919.
9. 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(2):104127.
10. Gurau AM, Mihalache D, Satala CB, Rață AM, Rebegea LF. HER2-Low Breast Cancer: Biological Framework and Determinants of HER2 Instability. Medicina (Kaunas). 2026;62(2):304.
11. College of American Pathologists. Reporting Template for Reporting Results of Biomarker Testing of Specimens from Patients with Carcinoma of the Breast. Version 1.6.1.0. Northfield, IL: College of American Pathologists; 2025 June. Available from: https://www.cap.org/wp-content/uploads/2026/07/Breast.Bmk_1.6.1.0.-REL_CAPCP-1.pdf
12. Tan GC, Alip A, Ho ICV, et al. Guidelines for HER2 reporting in breast cancer: Recommendations by the Malaysian Breast Pathology Working Group. Malays J Pathol. 2025;47(3):347-353.