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REVIEW

HER2 Heterogeneity in Breast Cancer: Biological Basis, Clinical Implications, and Therapeutic Strategies

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Abstract: Human epidermal growth factor receptor 2 (HER2) is a key driver of breast cancer and exhibits significant heterogeneity at the temporal, spatial, and molecular levels. This heterogeneity not only complicates HER2 assessment but also significantly impacts the efficacy of targeted therapies, ultimately contributing to drug resistance. This review summarizes current standards for HER2 testing and highlights their inherent limitations. It outlines the multidimensional manifestations of HER2 heterogeneity and examines the underlying biological mechanisms driving this phenomenon. In addition, emerging diagnostic strategies and therapeutic approaches are being developed specifically to address HER2 heterogeneity. The review aims to provide insights for optimizing patient stratification and advancing precision treatment in breast cancer.

Keywords: breast carcinoma, human epidermal growth factor receptor 2 heterogeneity, targeted therapy, drug resistance

Introduction

Worldwide, breast cancer represents the most frequently diagnosed cancer in women and a leading cause of cancer-related mortality.[1] Human epidermal growth factor receptor 2 (HER2) is an important driver gene in breast cancer initiation and progression. HER2 amplification or protein overexpression is closely associated with enhanced tumor invasiveness, increased metastatic risk, and poor prognosis.[2] The application of HER2-targeted agents has substantially prolonged survival among patients with HER2-positive breast cancer. Furthermore, antibody-drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) has expanded the population benefiting from treatment to include patients with HER2-low tumors.[3]

However, HER2 exhibits complex heterogeneity within tumors and across different lesions, including uneven gene amplification, differential protein expression, and dynamic evolution induced by treatment.[4] HER2 intratumoral heterogeneity (HER2-ITH) refers to the presence of tumor cell subpopulations exhibiting heterogeneous HER2 gene or protein expression within the same tumor. It can be classified into genetic ITH, caused by differences in ERBB2 gene copy number, and non-genetic ITH, which occurs on a background of uniform gene amplification.[4–6] A significant association has been demonstrated between HER2 heterogeneity and both diminished therapeutic response and poorer long-term survival following targeted therapy.[7–9]

In the era of ADC therapy, HER2 is increasingly regarded as a biomarker with a continuous distribution and dynamic variability.[10] Changes in HER2 expression between primary and metastatic or recurrent lesions, as well as before and after neoadjuvant therapy, further exacerbate its spatiotemporal heterogeneity. This complexity poses new challenges for HER2 testing, patient stratification, and treatment decisions. Accordingly, this review summarizes the manifestations and

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underlying mechanisms of HER2 heterogeneity and further discusses its clinical implications and potential management strategies.

Clinical Standards and Current Practices of HER2 Testing

Early clinical trials of anti-HER2 targeted therapies revealed substantial inter-laboratory variability in HER2 test results. To address this issue, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) issued the first standardized guidelines for HER2 testing and interpretation in 2007. Immunohistochemistry (IHC) is used to assess protein expression levels, while in situ hybridization (ISH) is employed to evaluate ERBB2 gene amplification in cases with IHC 2+.[11] The 2013 update further refined the interpretation criteria for IHC and ISH, providing a clearer definition for IHC 2+ cases and standardizing the classification of ISH results.[12] This revision improved the accuracy of HER2 testing. The 2018 update focused on rare scenarios and challenging borderline results encountered in clinical practice.[13]

In recent years, the DESTINY-Breast04 and DESTINY-Breast06 trials have demonstrated clinically meaningful treatment benefits in patients with HER2-low and HER2-ultralow expression, respectively. The traditional binary classification of HER2 as positive or negative may no longer fully meet the needs of precise patient stratification in the era of ADCs. Consequently, the 2023 ASCO/CAP guidelines emphasized the importance of distinguishing IHC 0 from IHC 1+. However, HER2-null, HER2-ultralow, and HER2-low have not yet been recognized as distinct biological subtypes.[14] (Figure 1 and Table 1). According to these criteria, tumors with IHC 3+ or IHC 2+/ISH amplified results are defined as HER2-positive, whereas IHC 0 or 1+ is considered HER2-negative.[12,13]

Despite continuous refinement of current testing standards, determination of HER2 status remains subject to inter-observer variability and sampling bias. Pre-analytical variables, such as tissue fixation, antigen retrieval, antibody clone, and assay conditions, can influence HER2 staining intensity and ISH sensitivity.[15,16] Prolonged cold ischemia time can attenuate the signal intensity of HER2 IHC staining,[17] while storing paraffin-embedded sections for excessive periods

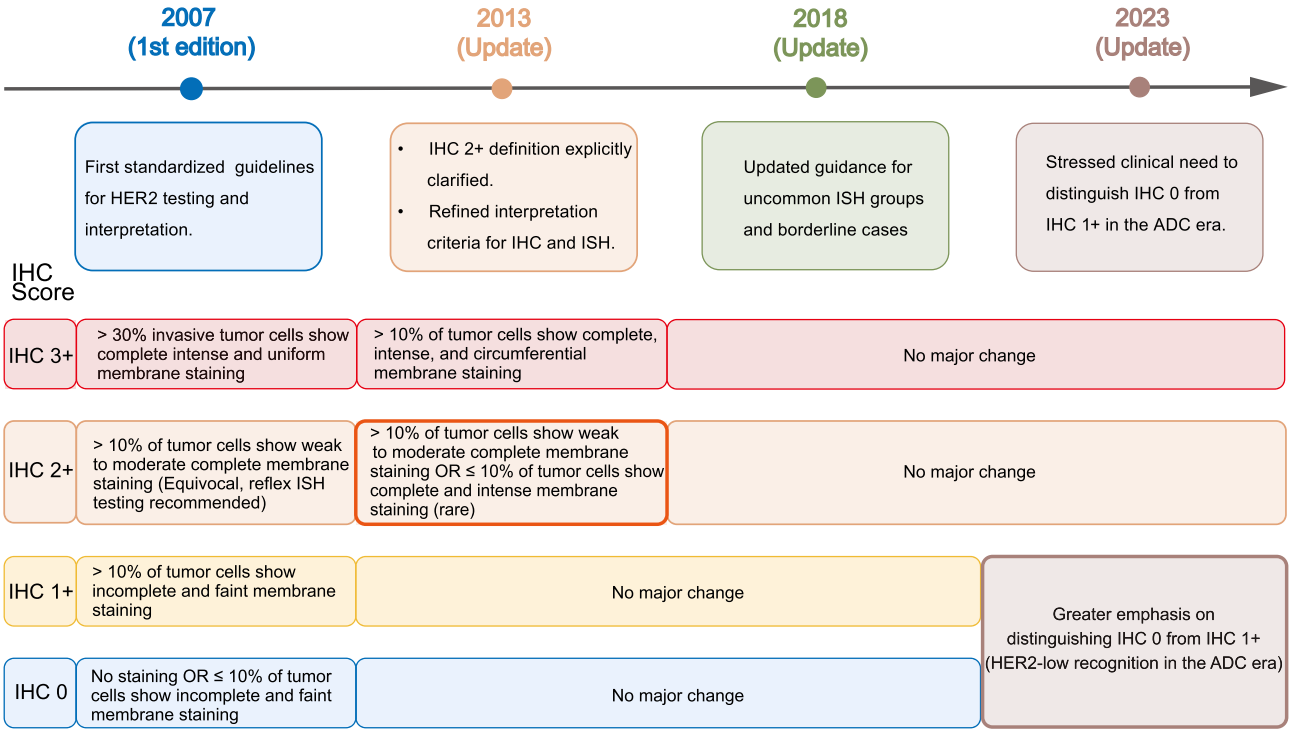

Figure 1 Evolution of ASCO/CAP HER2 IHC interpretation guidelines from 2007 to 2023. The figure highlights major updates in HER2 IHC interpretation. Bold borders indicate the two most significant changes in guideline evolution: refinement of the IHC 2+ category in the 2013 update and increased clinical emphasis on distinguishing IHC 0 from IHC 1+ in the ADC era (2023 update). Overall, the evolution reflects a shift from traditional binary HER2 classification toward a continuous HER2 expression spectrum.

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Table 1 HER2 ISH Interpretation Based on the 2018 ASCO/CAP Updated Guidelines

ISH Group	HER2/CEP17 Ratio	Average HER2 Copy Number per Cell	Interpretation
Group 1	≥2.0	≥4.0	Positive
Group 2	≥2.0	<4.0	Requires correlation with IHC
Group 3	<2.0	≥6.0	Requires correlation with IHC
Group 4	<2.0	≥4.0 and <6.0	Requires correlation with IHC
Group 5	<2.0	<4.0	Negative

may lead to a decline in the HER2 positivity detection rate.[18] Insufficient (< 6 hours) or excessive (> 72 hours) fixation increases the risk of errors in interpretation.[12] Furthermore, detection antibodies differ in specificity and sensitivity, with HercepTest and PATHWAY 4B5 representing two commonly used assays.[19,20]

Multiple real-world studies have indicated considerable variability in the assessment of HER2-zero, HER2-ultralow, and HER2-low expression. A multicenter study reported that among 170 breast cancer samples interpreted by 18 pathologists, the concordance rate for IHC 1+ interpretations was less than 1%. The overall concordance rate for IHC 0 cases was only 25%.[21] When IHC 0 was further subdivided into null and ultralow expression, agreement declined further (Fleiss $\kappa = 0.230$), markedly lower than that observed when classified simply as IHC 0 (Fleiss $\kappa = 0.344$).[22] Additionally, the proportion of HER2-low cases reported by different pathology departments varied substantially, ranging from 46.3% to 71.8% ($P < 0.0001$).[23] This variability is partly attributable to the continuous biological spectrum of HER2 expression, with most tumors exhibiting HER2/CEP17 ratios between 1 and 2, where conventional methods are limited in distinguishing extremely low expression levels.[24] Although overall ISH concordance is generally high, it decreases significantly in cases with signals near the cutoff or in tumors exhibiting genetic heterogeneity.[25]

Multidimensional Manifestations of HER2 Heterogeneity

Spatial Heterogeneity of HER2

HER2 expression in breast cancer exhibits substantial variability across different spatial levels. Such heterogeneity may arise within distinct regions of the primary tumor, between primary and metastatic lesions, and among different metastatic sites.[26–28] In a large paired sample analysis, comparison of 1299 matched primary and metastatic tumors revealed that 28.5% of patients experienced a change in HER2 status upon disease recurrence or metastasis, with the most common transition being from HER2-zero to HER2-low expression.[29] Collectively, these results indicate that HER2 expression undergoes dynamic changes over the course of disease progression. In addition to tissue biopsy, liquid biopsy has also provided evidence from a dynamic perspective supporting the presence of spatial heterogeneity.[30]

Spatial discordance is more pronounced in the HER2-low population. Wu re-stratified HER2-negative cases based on HER2-zero, HER2-ultralow, and HER2-low expression. They found that 23.7% of cases exhibited discordant HER2 status across different lesions. In 12.0% of cases, the index lesion was not the site with the highest HER2 expression.[31] Under current guidelines, HER2 testing is typically performed on the primary or a representative lesion. However, this approach may lead to underestimation of HER2 expression in some patients, potentially depriving them of the opportunity to receive ADC therapy. Furthermore, an autopsy study provided the first systematic evidence of marked spatial heterogeneity in HER2-low expression. The results showed that 80% of patients harbored both HER2-low and HER2-zero metastatic lesions. The proportion of HER2-low lesions varied widely among individuals, ranging from 5% to 89%.[32]

These findings challenge the conventional paradigm of relying on a single metastatic biopsy to guide treatment decisions. They support repeat biopsies at recurrence or metastasis, and integrating multi-regional sampling or liquid biopsy may further improve the accuracy of HER2 assessment and optimize therapeutic decision-making.

Dynamic Evolution Under Therapeutic Selection Pressure

In the context of neoadjuvant therapy for breast cancer, HER2 expression is not stable before and after treatment. Following neoadjuvant chemotherapy (NAC), 23.7% of cases exhibited discordant HER2 status between core needle

biopsy (CNB) and surgical specimens. By comparison, patients who did not receive NAC showed a lower rate of discordance (12.5%, P = 0.023). Among the NAC cohort, approximately 3.4% of HER2-positive patients converted to HER2-negative status after receiving trastuzumab.[33] Several studies have consistently demonstrated the loss of HER2 gene amplification following anti-HER2 targeted therapy.[34–36] Therapeutic pressure may select for HER2-negative clones, leading to the loss of high HER2 expression and subsequently influencing treatment response.[37] In patients with HER2-low tumors without a pathological complete response (PFS) after neoadjuvant therapy, a discordance rate of 26.4% was observed between pre- and post-treatment evaluations. Bidirectional conversion occurred in 33.8% of patients with baseline HER2-zero expression and in 37.7% of those with baseline HER2-low expression.[38] A study by Kang reported an overall discordance rate as high as 36.5% in a larger cohort, further emphasizing the marked instability of HER2-low expression during treatment.

This instability varies across different molecular subtypes. In HER2-negative patients, the hormone receptor (HR)-positive subgroup shows a significantly higher proportion of HER2-low expression compared with the HR-negative subgroup (68.6% vs 46.8%, P = 0.001).[37] HR-positive patients are also more likely to acquire HER2-low expression after treatment (OR = 2.48, P < 0.001).[39] The study by Lian reinforced this association. Among patients with baseline HER2-low expression, the HR-positive subgroup exhibited the highest phenotypic stability (80.82% remained stable), while HR-negative patients were more likely to undergo expression conversion.[40] These results suggest that HER2-low expression has a relatively high conversion rate across patients. However, in the presence of active HR signaling, the regulation of HR-related transcriptional networks may promote the maintenance or acquisition of the HER2-low expression phenotype.

Molecular Heterogeneity of HER2

Current understanding of HER2 molecular heterogeneity has become increasingly well characterized, and its underlying biological mechanisms are being elucidated. Multi-omics analyses have classified HER2-positive breast cancer into subtypes with distinct biological features and therapeutic sensitivities, including the classical target-responsive subtype, immune-modulatory subtype, luminal-like subtype, and basal/mesenchymal-like subtype.[41,42] Similarly, based on the expression patterns of genes related to histone modifications, HER2-low breast cancer exhibits three distinct subtypes that differ in prognosis and pathway activation.[43] These classifications indicate that significant molecular heterogeneity exists between tumors, even within the same HER2 expression category.

HER2-ITH manifests as multidimensional differences at the genomic, protein expression, and cellular state levels. When HER2 expression approaches the diagnostic threshold or the HER2/CEP17 ratio is elevated, heterogeneity in HER2 gene amplification becomes more readily detectable.[44] This genomic heterogeneity provides a foundation for differences in protein expression. At the protein spatial level, HER2-ITH exhibits three histological patterns. (Figure 2) The clustered pattern is characterized by the simultaneous presence of HER2-high (IHC 3+) and HER2-negative/low

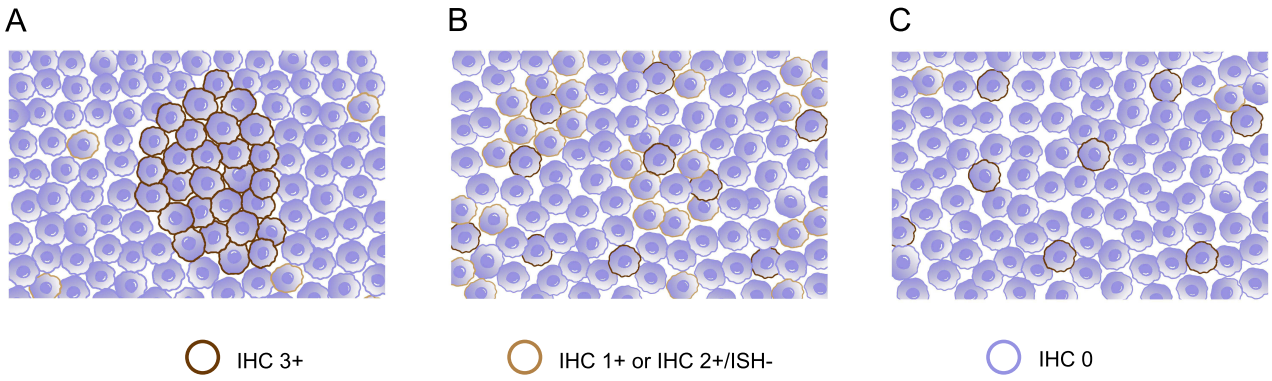

Figure 2 Schematic diagram of HER2 intratumoral heterogeneity patterns by immunohistochemistry. This schematic illustrates HER2-ITH in breast cancer, showing three distribution patterns of tumor cells. Clustered pattern (**A**): HER2-high cells form contiguous clusters. Mosaic pattern (**B**): HER2-high and HER2-low/negative cells are intermixed without clear boundaries. Scattered pattern (**C**): Rare HER2-high cells are dispersed in predominantly HER2-low/negative regions.

(IHC 0/1+) tumor cells, with a distinct boundary between them. The mosaic pattern manifests as a diffuse intermingling of cells with differing HER2 expression levels, whereas the scattered pattern consists of a tumor cell population predominantly composed of HER2-negative/low cells, interspersed with isolated HER2-high cells.[45,46] Moreover, in some HER2-positive tumors, HER2 protein expression does not occur in isolation but varies coordinately with markers such as estrogen receptor (ER), forming a continuous expression spectrum at the single-cell level.[47,48] The heterogeneity across genomic, protein, and cellular states underlies dynamic changes in HER2 expression and differential responses to therapy.

Clinical Cost of Heterogeneity: Variations in Treatment Efficacy and Origins of Drug Resistance

Heterogeneity in Therapeutic Response Caused by HER2 Heterogeneity

Previous studies have indicated that the HER2-enriched subtype is associated with higher pCR rates and better long-term survival outcomes in patients receiving neoadjuvant HER2-targeted therapy.[49,50] In contrast, tumor heterogeneity is linked to reduced therapeutic benefit and is a major driver of resistance to targeted therapy.[7,51,52] Multiple studies have demonstrated that a high level of HER2-ITH is an independent factor associated with lower pCR rates after NAC combined with anti-HER2 therapy.[8,52] A prospective Phase II trial of neoadjuvant trastuzumab emtansine (T-DM1) plus pertuzumab revealed a marked disparity in pCR rates according to HER2-ITH status, with 0% in patients with HER2-ITH compared with 55% in those without ($P < 0.0001$).[53] Evidence from mouse models and single-cell analyses also associates high heterogeneity with shorter disease-free survival (DFS) and a higher risk of disease progression.[9,54]

Within the traditional binary HER2 classification framework, differences in HER2 expression levels influence treatment response. Pooled analyses from multiple studies and prospective trials have shown that HER2-low breast cancer has an inferior pCR rate compared with HER2-zero disease following NAC.[55–58] Direct prospective clinical evidence comparing long-term survival outcomes among these groups remains limited, while retrospective studies have yielded inconsistent results. Although HER2-low serves as a therapeutic target for ADC, its independent prognostic value is yet to be established. In HER2-positive patients, tumors with IHC 2+/ISH-amplified status exhibit lower pCR rates than those with IHC 3+ tumors. These tumors also contain a smaller proportion of the HER2-enriched subtype, potentially limiting the efficacy of targeted therapy in this subgroup.[59,60]

Dynamic changes in HER2 expression further impact clinical outcomes. Patients with loss of HER2 expression following neoadjuvant therapy had a significantly increased risk of recurrence (HR 1.85, 95% CI: 1.31–2.61, $P = 0.0005$) and poorer overall survival (OS) (HR 2.37, 95% CI: 1.27–4.41, $P = 0.0065$) relative to those with stable HER2 status.[61] Spatiotemporal heterogeneity between primary and metastatic lesions also predicts treatment response. Patients with consistently HER2-positive disease achieved a median progression-free survival (PFS) of 16.8 months, significantly longer than the 13.7 months observed in those with acquired HER2 positivity or the 3.6 months in those who converted to HER2-negative ($P < 0.0001$).[62]

The Root Causes of Drug Resistance Induced by HER2 Heterogeneity

Anti-HER2 monoclonal antibodies (mAbs) and ADCs are the standard treatments for HER2-positive breast cancer in both neoadjuvant and adjuvant settings.[63,64] Although these therapies improve survival outcomes for most patients, resistance related to HER2 heterogeneity remains a major challenge.

Compared with tumors exhibiting homogeneous HER2 overexpression, tumors with HER2 heterogeneity typically display lower ERBB2 mRNA and HER2 protein levels, resulting in a "target dilution" effect that reduces the binding and internalization of antibodies or ADCs.[34] Although HER2 expression or dependency may be decreased, certain tumors retain active downstream signaling pathways, including PI3K/AKT/mTOR, thereby offering alternative means of survival.[65,66] In parallel, compensatory activation of bypass pathways, including Insulin-like Growth Factor 1 Receptor (IGF-1R), Fibroblast Growth Factor Receptor (FGFR), and integrins, may further enhance tumor adaptability to targeted therapy.[67–69] Additionally, HER2 molecular variants contribute to therapeutic resistance. For instance, the

extracellular domain-truncated form p95HER2 lacks the trastuzumab-binding epitope and can mediate resistance to specific anti-HER2 therapies.[70]

Evidence suggests that treatment itself can drive clonal selection based on HER2 status.[37] Following therapy with T-DM1 plus pertuzumab, a subset of HER2-positive tumor cells is selectively eliminated, whereas less sensitive HER2-negative cells persist and expand, thereby increasing intratumoral heterogeneity.[53] Tumors with HER2 heterogeneity show limited overall transcriptomic changes after treatment, suggesting an intrinsic adaptive equilibrium state.[34]

The immunosuppressive tumor microenvironment (TME) is a major driver of resistance to HER2-targeted therapy. Multiple immune cell populations, including exhausted T cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), cooperatively impair antitumor immunity and limit treatment efficacy.[71] Tumor-infiltrating lymphocytes (TILs) in HER2-positive breast cancer frequently display an exhausted phenotype. In early-stage disease, multiple checkpoint receptors, including PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT, are co-expressed on CD8+cytotoxic T cells and CD16+CD56bright natural killer (NK) cells. This coordinated checkpoint activation suppresses cytotoxic antitumor responses and facilitates immune evasion.[72]

MDSCs are central mediators of immune suppression within the TME. Pro-inflammatory cytokines such as IL-6 and IL-8 activate NF-κB and IL-6/STAT3 signaling, thereby promoting MDSC expansion and activation.[73,74] IL-6 derived from tumor-infiltrating NK cells correlates with Arg1 expression in MDSCs. Inhibition of the IL-6/STAT3 axis alleviates T-cell suppression and reduces tumor growth.[75] MDSCs further suppress T-cell function through Arg1 secretion and PD-L1 upregulation, ultimately compromising antibody-dependent cellular cytotoxicity (ADCC).[74] Collectively, these findings highlight the critical role of myeloid-mediated immune suppression in resistance to HER2-targeted therapy.

TAMs are another major immunosuppressive component of the TME. They are generally classified into the tumoricidal M1 subtype and the immunosuppressive M2 subtype, the latter representing the dominant phenotype associated with tumor-promoting and immunosuppressive functions.[76] TAM-derived IL-10 activates STAT3/Bcl-2 signaling and promotes chemoresistance in breast cancer cells.[77] In parallel, TAM-derived IL-8 enhances EGFR-related signaling and contributes to lapatinib resistance.[78] TAMs additionally suppress $CD8^+$T-cell activity through PD-L1 upregulation and subsequent interaction with PD-1. Sustained PD-1/PD-L1 signaling may eventually result in T-cell exhaustion.[79] TAMs also participate in antibody-dependent cellular phagocytosis (ADCP), thereby modulating trastuzumab efficacy. Although ADCP mediates antibody-dependent phagocytosis of tumor cells and exerts antitumor effects, it may also attenuate this response by promoting PD-L1 upregulation on TAMs.[80,81] Furthermore, TAMs regulate stromal cell function through signaling pathways such as STAT3/NF-κB, indirectly promoting M2 macrophage-mediated chemoresistance.[82]

Diagnostic Upgrades and Therapeutic Strategies for HER2 Heterogeneity

Diagnostic Upgrades

Given the dynamic nature of HER2 expression, serial tumor biopsies are recommended at baseline, after treatment, as well as at recurrence and in the metastatic setting, to assess changes in molecular characteristics.[83] As an invasive procedure, CNB reflects only the molecular state of a localized tumor region and may not fully capture the biological features of the overall tumor burden. In contrast, as a non-invasive imaging technique, positron emission tomography/computed tomography (PET/CT) enables the assessment of HER2 expression throughout the body, providing valuable information for clinical treatment decisions and patient management.[84]

In patients with advanced HER2-positive breast cancer, ^{89}Zr-trastuzumab PET effectively identifies lesions unresponsive to T-DM1 therapy, demonstrating a negative predictive value (NPV) of 81%. When combined with early metabolic imaging via FDG PET/CT, the NPV for non-response to treatment increases to 100%. Moreover, the median time to treatment failure in patients with negative HER2 PET findings is significantly shorter (2.8 vs 9.9 months; HR = 3.7; 95% CI: 2.19–6.35; $P < 0.0001$).[85] The IMPACT-MBC study, which enrolled patients with all subtypes of metastatic breast cancer, revealed significant systemic heterogeneity in HER2 expression. From the data obtained, an algorithm was developed to predict IHC positivity, achieving an area under the curve (AUC) of 0.86 (95% CI: 0.79–0.93).[86] This whole-body visualization of HER2 expression challenges the representativeness of conventional single-site CNB in capturing

tumor heterogeneity. Preliminary investigations using novel molecular tracers like ^{68}Ga-ABY-025 have demonstrated that HER2 PET imaging is both feasible and safe, and can also uncover inter-lesion heterogeneity in tracer uptake.[87]

Artificial intelligence (AI) technologies are being applied in pathological image analysis. By leveraging image recognition algorithms, AI enables the quantitative analysis of HER2 membrane staining, thereby yielding more objective, accurate, and reproducible diagnostic results. This technology holds significant potential for aiding pathological diagnosis.[88,89] Deep learning-based algorithms can automatically detect and classify interphase nuclei and HER2/CEP17 signals in ISH images. Their diagnostic accuracy is comparable to that of a team of pathologists, with an overall concordance of 96%. At the single-nucleus level, the Cohen's κ coefficient for classification approaches the level observed between pathologists.[90] Wu and colleagues provided direct evidence for the application of AI in standardizing HER2-low and heterogeneous IHC scoring. By integrating AI-assisted interpretation with an augmented reality microscope system, overall scoring accuracy improved from 0.80 to 0.93, and inter-observer agreement increased from 0.542 (95% CI: 0.496–0.592) to 0.812 (95% CI: 0.783–0.840).[91] In the future, AI is expected to serve as a valuable adjunct to pathologists, ultimately improving patient stratification for HER2-targeted therapies and enhancing treatment outcomes.

Beyond improving diagnostic accuracy, AI-assisted HER2 scoring has the potential to directly inform personalized treatment strategies. A systematic review and meta-analysis evaluated the ability of AI to distinguish HER2 IHC scores, using IHC 1+/2+/3+ as the positive threshold for T-DXd eligibility. The results demonstrated a pooled sensitivity of 0.97 (95% CI, 0.96–0.98), a pooled specificity of 0.82 (95% CI, 0.73–0.88), and an area under the curve of 0.98 (95% CI, 0.96–0.99).[92] This indicates that among 100 patients with metastatic breast cancer, AI would miss only three patients who could potentially benefit from T-DXd therapy, while eighteen patients might be overtreated.

Unlike diagnostic models that rely solely on IHC scoring, deep learning-based models can integrate multimodal imaging data to overcome sampling bias caused by intratumoral heterogeneity in CNB specimens, thereby enabling noninvasive prediction of neoadjuvant treatment response. Zhang developed a multimodal alignment and prediction (MAP) model using multicenter datasets that integrate imaging and clinical information. The model achieved 79% concordance between predicted HER2 status and surgical specimens, substantially exceeding the approximately 41% concordance observed between CNB and surgical specimens. These findings demonstrate that AI can effectively address HER2 spatial heterogeneity by capturing the whole-tumor field rather than a localized biopsy sample.[93] Furthermore, the MAP model was also applied to predict pCR following neoadjuvant therapy. In the internal test cohort, the AUC for treatment response prediction based on AI-predicted HER2 status reached 0.858, significantly outperforming the AUC of 0.826 derived from biopsy-based HER2 assessment ($P < 0.05$).[93] Similarly, another study developed an AI-based efficacy prediction model for SHR-A1811, which also demonstrated robust performance in a real-world multi-ADC cohort including T-DXd, SHR-A1811, and TQB2102 (AUC = 0.83, 95% CI: 0.68–1.00). This multi-modal learning framework generalizes across next-generation ADCs, providing predictive capability for both single and multiple agents.[94] Although AI-assisted HER2 assessment requires further validation in larger prospective studies, its preliminary success in improving diagnostic consistency, predicting treatment response, and guiding individualized ADC therapy has substantially advanced the clinical translation of precision medicine.

Therapeutic Strategies for HER2 Heterogeneity

The dual HER2-targeted regimen combining trastuzumab and pertuzumab has become a cornerstone in the treatment of HER2-positive breast cancer based on evidence from multiple pivotal clinical trials. In the phase II NeoSphere study, the addition of pertuzumab to standard neoadjuvant chemotherapy plus trastuzumab increased the pCR rate to 45.8% (95% CI: 36.1–55.7), outperforming trastuzumab plus chemotherapy alone.[95] Long-term follow-up of the APHINITY trial in patients with early HER2-positive breast cancer receiving adjuvant chemotherapy plus dual HER2 blockade demonstrated a significant improvement in invasive disease-free survival (iDFS) in the overall population. In the pertuzumab group, the 6-year iDFS rate was 91%, compared with 88% in the control group (HR 0.76; 95% CI: 0.64–0.91).[96] The CLEOPATRA study established dual HER2 blockade combined with chemotherapy as the standard of care for metastatic disease, with a median OS of 57.1 months (95% CI: 50–72).[97] This therapeutic advantage is primarily attributed to the complementary mechanisms of the two antibodies, which bind to distinct extracellular domains of the HER2. Such dual

blockade achieves more potent inhibition of HER2 dimerization and downstream signaling.[98,99] Multi-epitope strategy confers the potential to overcome the heterogeneity of HER2 expression.

In recent years, the emergence of ADCs has provided a novel therapeutic strategy to overcome HER2 heterogeneity. ADCs deliver cytotoxic agents into tumor cells through monoclonal antibody-mediated specific binding. Following internalization and lysosomal degradation, the cytotoxic payload is released to exert antitumor effects.[100,101] Notably, some payload molecules can diffuse into adjacent cells and induce cell death, which is known as the bystander effect.[102] This characteristic enables ADCs to exert a relatively broad antitumor effect even in the tumor microenvironments with heterogeneous HER2 expression.

T-DXd is one of the most representative HER2-targeted ADCs. Compared with T-DM1, T-DXd achieved significantly longer PFS in patients with HER2-positive metastatic breast cancer previously treated with trastuzumab and taxanes. Median PFS was 29.0 months in the T-DXd group versus 7.2 months in the T-DM1 group (HR = 0.30; 95% CI: 0.24–0.38). Median OS also favored T-DXd (52.6 vs 42.7 months; HR = 0.73; 95% CI: 0.56–0.94). Both the objective response rate (ORR) and PFS were markedly superior with T-DXd compared to T-DM1.[103] The DESTINY-Breast04 trial was the first to demonstrate that HER2-low metastatic breast cancer represents a distinct population that can derive significant benefit from ADC therapy. This Phase III study showed that, compared with conventional chemotherapy, T-DXd significantly prolonged both PFS (median PFS: 9.9 vs 5.1 months; HR = 0.50; $P < 0.001$) and OS (median OS: 23.4 vs 16.8 months; HR = 0.64; $P = 0.001$).[3] The efficacy of T-DXd in HER2-low tumors is thought to be attributable to its unique ADC design. A high-affinity antibody ensures effective targeting even at low antigen density, while the combination of a cleavable linker and a membrane-permeable cytotoxic payload enhances the bystander effect. Together, these features expand the cytotoxic range and overcome intratumoral HER2 heterogeneity.[3,104]

Subsequently, the DESTINY-Breast06 trial further extended the clinical application of T-DXd to patients with HER2-ultralow tumors. HER2-ultralow is defined as a subset of IHC 0 tumors showing faint but detectable membranous staining (>0%).[105] In this subgroup, T-DXd treatment continued to provide clinically meaningful benefit, with a PFS of approximately 13.2 months (95% CI, 9.8–17.3) and an ORR of 61.8% (95% CI, 50.0–72.8).[106] Although DESTINY-Breast06 independently confirmed that HER2-ultralow patients may benefit from ADC therapy, the trial did not include HER2-null patients as a separate analysis cohort. Recently, CAP issued reporting templates recommending further subdivision of conventional IHC 0 tumors into HER2-null and HER2-ultralow. This approach is expected to more precisely identify patients most likely to benefit from ADC treatment.[105] Furthermore, the enrollment periods of these key clinical trials spanned several years. During this time, multiple updates to the ASCO/CAP guidelines were issued, and HER2 scoring criteria continued to evolve. This evolution may, to some extent, affect the comparability of patient populations across studies, a factor that should be considered when integrating and analyzing relevant data.

Beyond optimizing HER2-targeted therapies, developing treatment strategies independent of HER2 expression represents an important approach to addressing HER2 heterogeneity. Regardless of HER2 expression, ADCs targeting Trop-2, such as sacituzumab govitecan (SG) and datopotamab deruxtecan (Dato-DXd), are representative agents. By binding to the widely expressed Trop-2 protein on tumor cells and delivering cytotoxic payloads, these agents exert potent antitumor effects.[107,108] Such ADCs may eliminate both HER2-positive and HER2-negative clones simultaneously, offering a potential alternative for tumors that are resistant to HER2-targeted therapies due to intratumoral heterogeneity.

Faced with the complex drug-resistance landscape posed by HER2 heterogeneity, traditional single-target strategies often prove inadequate. Multi-targeted approaches have increasingly become a focus of research. Zanidatamab is a HER2-directed IgG1 bispecific antibody that simultaneously binds to two distinct domains, ECD4 and ECD2, on the HER2 receptor.[109] This dual-epitope targeting not only more effectively inhibits HER2 signaling but also induces HER2 receptor clustering and internalization, while enhancing ADCC and ADCP.[110] In clinical studies targeting unresectable, locally advanced, recurrent, or metastatic HER2-positive breast cancer, zanidatamab and docetaxel demonstrated substantial antitumor activity. The combination yielded an ORR of 90.9% (95% CI: 75.7–98.1%) and a disease control rate (DCR) of 97.0% (95% CI: 84.2–99.9%), with a median duration of response of 23.5 months (95% CI: 11.3–NE). Median PFS reached 22.1 months (95% CI: 12.7–NE), while median OS was 36.9 months (95% CI: 36.9–NE).[111] Another randomized clinical trial (NCT04224272) investigated zanidatamab combined with pembrolizumab and fulvestrant in

patients with HR-positive/HER2-positive breast cancer, achieving a 6-month PFS rate of 66.7% (95% CI: 52.1–79.2%).[112] These observations support multi-epitope HER2 targeting as a promising strategy to circumvent HER2 signaling heterogeneity.

By binding to the ATP-binding site of the HER2 intracellular tyrosine kinase domain, small-molecule tyrosine kinase inhibitors (TKIs) block receptor autophosphorylation and disrupt downstream signaling pathways, including PI3K/AKT and MAPK.[113] Their antitumor activity is not fully dependent on HER2 membrane expression levels, suggesting that TKIs may provide more consistent inhibition in tumors with heterogeneous HER2 expression.[113,114] Lapatinib, a first-generation reversible dual HER1/HER2 TKI, was the first agent to demonstrate the clinical feasibility of targeting the intracellular HER2 kinase domain. However, its reversible binding properties result in limited durability of HER signaling inhibition, which may partially restrict its therapeutic activity in tumors with heterogeneous HER2 expression.[115] In contrast, the irreversible pan-HER TKIs neratinib and pyrotinib achieve more sustained blockade of HER signaling through covalent binding while simultaneously expanding the spectrum of HER family receptor inhibition.[116] The phase III NALA trial demonstrated that neratinib plus capecitabine significantly reduced the risk of disease progression or death in patients with metastatic breast cancer who had received at least two prior lines of anti-HER2 therapy (HR = 0.76, 95% CI: 0.63–0.93; P = 0.0059).[117] Similarly, the PHOEBE trial showed that pyrotinib combined with capecitabine significantly improved PFS in patients previously treated with trastuzumab (median PFS: 12.5 vs 6.8 months; HR = 0.39; P < 0.0001).[118] These findings suggest that irreversible pan-HER inhibition may provide more durable pathway blockade in tumors with heterogeneous HER2 expression.

In the HER2CLIMB trial, the addition of tucatinib to trastuzumab and capecitabine yielded significantly improved outcomes for patients with previously treated HER2-positive metastatic breast cancer.[119] Both OS and PFS were significantly prolonged compared with the control group (trastuzumab plus capecitabine alone). The HER2CLIMB-02 study further evaluated tucatinib combined with T-DM1 in the population with locally advanced or metastatic HER2-positive disease.[120] Median PFS was superior in the tucatinib arm relative to the control arm (9.5 vs 7.4 months; HR = 0.76; 95% CI: 0.61–0.95; P = 0.0163). Integration of clinical findings with preclinical studies suggests that HER2 TKIs combined with trastuzumab or ADCs enhance antitumor activity through simultaneous blockade of both extracellular and intracellular HER2 signaling, resulting in a synergistic effect.[114,121,122] In tumors with heterogeneous HER2 expression, the drug combination may offer advantages in overcoming HER2 heterogeneity.

BL-B01D1 is a bispecific ADC targeting EGFR and HER3. Upon binding to EGFR or HER3 receptors on tumor cells, the ADC undergoes internalization and releases its cytotoxic payload, Ed-04, within lysosomes, where the released payload induces tumor cell death by inhibiting DNA replication and RNA synthesis.[123] Phase I clinical studies have demonstrated preliminary antitumor activity of BL-B01D1 in multiple solid tumor types.[124] Ongoing clinical trials are evaluating BL-B01D1 in patients with unresectable locally advanced or metastatic triple-negative breast cancer, and for HR-positive/HER2-negative breast cancer, with its efficacy yet to be established.

Significant progress has been made in the treatment of HER2-positive and HER2-low breast cancer over the past decade. However, high-quality clinical evidence directly supporting the efficacy of these therapeutic strategies in tumors with confirmed HER2 heterogeneity remains limited (Table 2). Most existing clinical studies have not incorporated HER2 heterogeneity as a stratification or selection criterion at enrollment, making it difficult to determine which strategy is most effective for heterogeneous tumors. At present, the efficacy of these treatments in HER2-heterogeneous tumors is largely derived from mechanistic speculation or indirect evidence observed in unselected populations without heterogeneity screening. A fundamental challenge is the lack of a unified pathological definition and standardized detection system for HER2 heterogeneity. This lack of standardization hinders accurate patient identification and complicates comparisons across studies. Future efforts should aim to establish standardized diagnostic and grading systems for HER2 heterogeneity. Incorporating these parameters as key stratification factors in prospective clinical trials will be essential to optimize patient selection and move toward truly precise, individualized treatment.

Conclusion

As a critical driver molecule in breast cancer, the expression status of HER2 has long served as a key determinant for patient classification and for guiding anti-HER2 therapy. Numerous studies indicate that HER2 expression exhibits

Table 2 Therapeutic Strategies to Overcome HER2 Heterogeneity in Breast Cancer

Therapeutic Strategy	Drug	Target	Mechanism	Study Population	Advantage
mAbs	Trastuzumab +Pertuzumab[95–97]	HER2-ECD4 /ECD2	Dual-epitope blockade; inhibition of HER2 dimerization	Early, unresectable, locally advanced, recurrent, or metastatic HER2-positive breast cancer	Enhanced HER2 blockade
HER2-targeted ADCs	T-DXd[3,103,106]	HER2	Delivery of cytotoxic payload; bystander effect mediated by membrane-permeable drugs	Metastatic HER2-positive and HER2-low breast cancer	Bystander effect
Non-HER2-targeted ADCs	SG[125]	Trop-2	Delivery of SN-38 payload; bystander effect	Metastatic triple-negative breast cancer	HER2-independent; bystander effect
Non-HER2-targeted ADCs	Dato-DXd[107]	Trop-2	Delivery of Deruxtecan payload; bystander effect	Metastatic triple-negative breast cancer	HER2-independent; bystander effect
BsAbs	Zanidatamab[111,112]	HER2-ECD4/ECD2	Dual-epitope blockade; induction of receptor Internalization; enhanced ADCC/ADCP	Advanced or metastatic HER2-positive breast cancer	Dual-epitope targeting; enhanced ADCC/ADCP
TKI-based combinations	Tucatinib + Trastuzumab + Capecitabine[119]	HER2-TK (Tucatinib) HER2-ECD4 (trastuzumab)	Inhibition of intracellular kinase signaling; blockade of extracellular receptor-mediated signaling; chemotherapy-induced DNA damage	Metastatic HER2-positive breast cancer	Multi-level HER2 blockade; efficacy in resistant disease
TKI-based combinations	Tucatinib + T-DM1[120]	HER2-TK (Tucatinib) HER2-ECD4 (T-DM1)	Inhibition of intracellular kinase signaling; ADC-mediated delivery of cytotoxic payload	Unresectable, locally advanced, recurrent, or metastatic HER2-positive breast cancer	Dual HER2 blockade; potential to overcome resistance
TKI-based combinations	Neratinib/ Pyrotinib +Capecitabine[117,118]	Pan-HER (HER1/HER2/ HER4) (Neratinib/ Pyrotinib)	Inhibition of intracellular pan-HER kinase signaling; chemotherapy-induced DNA damage	HER2-positive metastatic breast cancer with prior anti-HER2 therapy	HER2 expression-independent inhibition; efficacy in resistant disease
BsADCs	BL-B01D1[123,124]	EGFR HER3	Release of cytotoxic payload Ed-04 inhibition of DNA replication and RNA synthesis	Locally advanced or metastatic solid tumors	HER2-independent; potential activity in heterogeneous tumors

complex spatiotemporal and molecular heterogeneity in breast cancer. HER2 heterogeneity not only complicates accurate HER2 assessment but also contributes to substantial variability in treatment response and promotes therapeutic resistance. Further studies have revealed that HER2 heterogeneity affects the efficacy of anti-HER2 therapies through multiple mechanisms, including the target dilution effect, activation of bypass signaling pathways, and clonal selection.

A more comprehensive understanding of HER2 expression in tumors may be achieved by optimizing testing strategies, strengthening dynamic assessment at recurrence and metastasis, and incorporating emerging approaches such as liquid biopsy and molecular imaging. Meanwhile, the development of next-generation ADCs and multi-

targeted combination strategies offers new avenues to overcome resistance associated with HER2 heterogeneity. Integrating multidimensional molecular information and refining patient stratification systems will be critical to advancing precision medicine in breast cancer.

Disclosure

The authors report no conflicts of interest in this work.

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