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Review Article

HER2 Low Status and Intratumoral Heterogeneity in Epithelial Malignancies and Their Therapeutic Implications

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Abstract

Human epidermal growth factor receptor 2 (HER2) continues to serve as a critical biomarker in breast cancer, but the introduction of evolving classifications such as HER2-low and HER2-ultra-low presents both opportunities and challenges in precision oncology. Targeted therapies like trastuzumab deruxtecan have broadened treatment possibilities, yet these classifications highlight critical challenges related to standardization, diagnostic precision, and equal access to care. The heterogeneity of HER2 expression adds further complexity, often limiting therapeutic effectiveness. Additionally, the exploration of HER2 as a target in nonbreast cancers underscores the urgent need for rigorous clinical validation in diverse malignancies. To advance HER2-targeted therapies, there is a critical need for comprehensive research, improved diagnostic protocols, and strategies to ensure equitable access to innovative treatments.

Keywords
- HER2-low
- heterogeneity
- treatment strategies
- breast cancer
- trastuzumab deruxtecan

Introduction

Over the past two decades, there has been remarkable progress toward understanding human epidermal growth factor receptor 2 (HER2) as a significant prognostic marker in breast cancer (BC) along with deeper insight on its presence across different epithelial malignancies.[1,2] It is a tyrosine kinase receptor, encoded by a proto-oncogene ERBB2, which expresses itself by homo- or heterodimerization resulting in signal transduction mediated by the activation of PI3K/AKT and Ras/Raf/MEK/MAPK pathways that ultimately affects cell proliferation, survival, motility, and adhesion.[3] HER2 overexpression or amplification is associated with higher histologic grade and stage, increased metastatic potential, decreased overall survival, resistance to endocrine therapy, and poor response to selected chemotherapy.[4] Genetic and pharmacological studies have established that HER2 is essential and sufficient for tumor development and maintenance in models of HER2-amplified BC. Since HER2 amplification drives the transformed characteristics of neoplasms, direct pharmacological targeting of HER2 has been suggested.[5]

Current Clinical Practices for HER2 Assessment and Treatment in Breast Cancer

As a prognostic and predictive biomarker, HER2 status is routinely assessed by immunohistochemistry (IHC) and/or in situ hybridization (ISH) in BC. As per the 2018 American Society of Clinical Oncology (ASCO) guidelines,[6] BCs are classified as either HER2-positive (IHC3+ or 2+ with gene amplification by ISH) or HER2-negative (IHC 0+ or 1+ or 2+ without ISH amplification). Patients with HER2 positive disease typically have a worse prognosis with characteristics of aggressive tumor progress and shorter patient survival. The current HER2 targeting drugs include antibodies, tyrosine kinase inhibitors (TKIs) and antibody-drug conjugates (ADCs). Antibody-mediated therapy has been a highly effective strategy for treating

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epithelial malignancies over the past 30 years. With the introduction of trastuzumab, a recombinant monoclonal antibody, HER2-positive BC treatment was revolutionized. Currently, the Food and Drug Administration (FDA)-approved HER2 monoclonal antibodies for BC treatment include trastuzumab (Herceptin) and pertuzumab (Perjeta) that inhibit HER2 overamplification via downregulation of HER2-induced signaling or preventing its dimerization itself, respectively. However, resistance to HER2-targeted therapies, including trastuzumab, poses a significant challenge. TKI drugs block the phosphorylation of tyrosine kinase residues in the PI3K/AKT and MAPK pathways, which regulate tumor cell proliferation, migration, angiogenesis, drug resistance, and apoptosis. Lapatinib, pyrotinib, tucatinib, and neratinib are FDA-approved TKIs that reduce HER2 overexpression. ADCs provide a novel approach by selectively delivering cytotoxic drugs to HER2-expressing tumor cells. Combining HER2-directed agents with cytotoxic drugs has shown potential to overcome resistance and improve outcomes. Trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd) are antibody conjugates that received FDA approval for HER2 targeting.[7,8]

Emerging Insights into HER2 Clinico-Pathology

HER2-Low Status

The phase III DESTINY-Breast04 trial (NCT03734029) demonstrated the clinical actionability of HER2-low status, defined as HER2 expression of 1+ or 2+ by IHC without gene amplification, leading to the approval of T-DXd as the first HER2-targeted therapy for HER2-low BC. This has challenged the binary HER2 classification, paving the way for a ternary system: HER2-negative, HER2-low, and HER2-positive (►**Fig. 1**). HER2-low accounts for approximately 45 to 55% of all BC cases, with a higher prevalence in hormone receptor-positive (HR+) BC (55–65%) compared to triple-negative BC (TNBC, 35–40%). Additionally, the concept of HER2 ultra-low (IHC 0 with faint staining in ≤ 10% of tumor cells) has gained interest. HER2-low expression is emerging as a targetable biomarker, with significant therapeutic implications for HR+ BC and TNBC. However, questions remain regarding accurate assessment of HER2-low status, patient stratification, and its prognostic and biological significance. Further retrospective and prospective studies are required to refine the clinical and pathological understanding of HER2-low BC.[9]

HER2 Heterogeneity

HER2 intratumoral heterogeneity (ITH) is a well-known phenomenon in BC. The ASCO guidelines define HER2 heterogeneity as presence of a second population of tumor cells, where at least 10% have a different HER2 copy number and/or HER2/CEP17 ratio. A separate count of at least 20 nonoverlapping cells within this population must be performed and reported. The 2018 guidelines also recognized that unusual patterns of HER2 expression can occur, including strong and complete staining in fewer than 10% of tumor cells. Heterogeneous patterns of HER2 overexpression and amplification can be categorized into three types: the clustered type, where distinct tumor clones exhibit either HER2 amplification or normal HER2 status; the mosaic type, characterized by diffuse intermingling of cells with varying HER2 statuses; and the scattered type, where isolated HER2-amplified cells exist within a HER2-negative population. HER2 ITH contributes to variations in protein expression or gene amplification across different tumor regions, impacting prognosis and therapy outcomes. Studies have shown that HER2 ITH correlates with reduced disease-free survival in HER2-positive invasive BC and diminished trastuzumab efficacy in metastatic cases. While HER2 ITH is more common in HER2 IHC 2+/equivocal cases, recent findings highlight its notable presence in HER2-low tumors, emphasizing the need for further research.[10] HER2 ITH, characterized by the presence

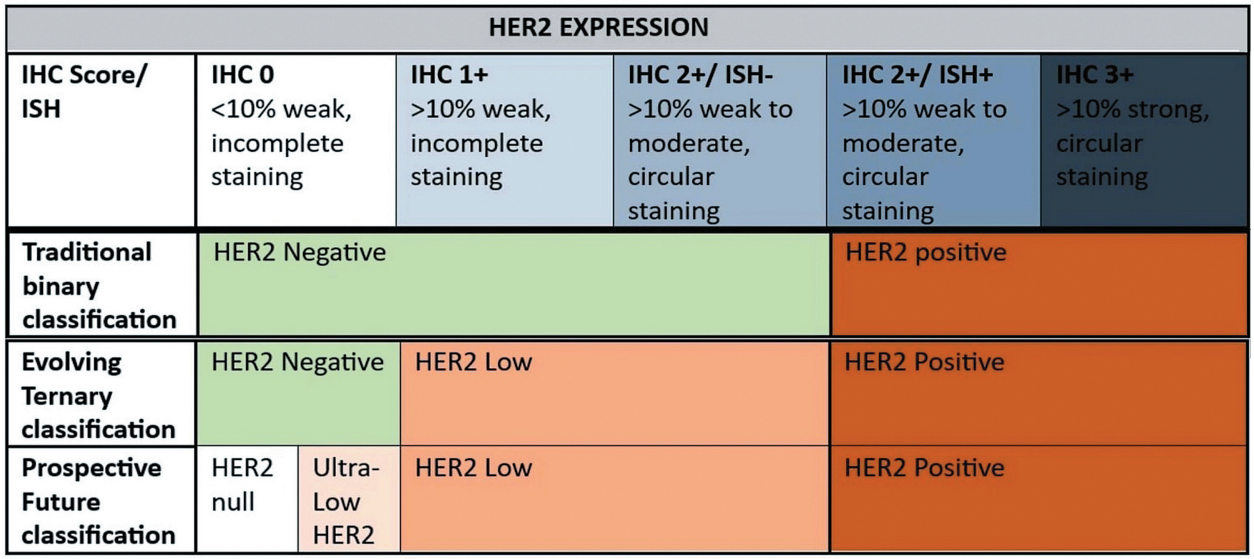

Fig. 1 Human epidermal growth factor receptor 2 (HER2) classification and current categories.

of at least two distinct cell clones with varying HER2 statuses within the same tumor, also poses significant challenges in accurately evaluating HER2 status in BC. HER2 heterogeneity, including both de novo and acquired resistance to anti-HER2 therapies like trastuzumab, presents significant hurdles in treating HER2-positive metastatic BC. It contributes to resistance by potentially causing inaccurate HER2 status assessment and inefficient drug targeting. Studies, such as the MARIANNE trial, show poorer performance of T-DM1 in patients with HER2 ITH compared to those with homogeneous HER2 expression. Patients with HER2 heterogeneity may require additional chemotherapy alongside anti-HER2 therapies, especially when ITH is significant.[11]

HER2 testing on small biopsies with ITH may not fully reflect tumor characteristics. Analyzing multiple slides or the entire slide, with separate ISH analysis for regions with differing HER2 IHC results, is recommended. ITH can cause discordance between IHC and ISH, impacting outcomes, especially in HER2-low patients. Advanced methods like the HER2 gene-protein assay, which combines IHC and ISH, could help detect HER2 microheterogeneity, which may drive resistance to targeted therapies. Hence, more efficient methods are needed to improve HER2 evaluations and therapy selection. Standardized criteria for HER2 discrepancies and ITH also need further investigation[12,13] (►**Fig. 2**).

Nonbreast Epithelial Malignancies

HER2 protein expression at a 3+ level, as identified through IHC, has been observed in a subset of nearly all carcinomas originating from epithelial tissues. HER2 positivity rates were reported as 64% in bladder carcinomas, 55% in gallbladder cancers, 22% in extrahepatic cholangiocarcinomas, 17% in cervical cancers, and around 21.3% in uterine cancers, comparable to the 30% HER2 overexpression rate seen in BC.[14–17] This highlights the potential of anti-HER2 therapies in nonbreast HER2-positive malignancies, having a meaningful impact on treatment strategies.[18]

In the global study of T-DXd for HER2-expressing solid tumors, it demonstrated promising objective response rate (ORR), particularly in IHC 3+ patients, along with durable clinical benefits and manageable side effects. Interim results suggest T-DXd as a potential treatment for HER2-expressing tumors. ORRs for various cancers were 57.5% (endometrial), 50% (cervical), 45% (ovarian), 39% (urothelial), 22% (biliary), and 4% (pancreatic).[19]

While HER2 is a proven predictive marker, its role as a prognostic factor in gastric cancer (GC) remains debated, with recent studies suggesting a negative impact on prognosis. HER2 overexpression in GC is associated with poor clinical outcome. Hence, inhibiting the HER family signal transduction is likely to contribute to improved survival of patients suffering from GC. The phase III ToGA trial demonstrated improved outcomes when trastuzumab was added to first-line fluoropyrimidine/platinum therapy in HER2-positive GC, establishing this combination as the standard of care.[20] HER2-targeted therapies, like trastuzumab, pembrolizumab, and T-DM1, have shown improved outcomes in HER2-positive cases. Emerging treatments, including margetuximab, zanidatamab, and KN026, demonstrate promise in clinical trials. Trastuzumab deruxtecan (T-DXd) and novel ADCs like ARX788 show significant efficacy in advanced settings, marking progress in HER2-positive GC treatment.[8]

HER2 overexpression in ovarian cancer varies widely (8–66%) and shows frequent ITH. While early anti-HER2 therapies like trastuzumab showed limited efficacy, newer drugs such as T-DXd have demonstrated promising outcomes, particularly in tumors with HER2 3+ scores. Preclinical and clinical studies have explored trastuzumab (Herceptin) and pertuzumab (Perjeta) in ovarian cancer, particularly HER2-amplified mucinous subtypes.[21] The DESTINY-PanTumor02 trial demonstrated significant efficacy of trastuzumab deruxtecan in HER2-expressing gynecological cancers, even in heavily pretreated patients, suggesting its potential as a tumor-agnostic therapy.[19,22] Phase II trials with trastuzumab and lapatinib showed limited responses in ovarian cancer, though trastuzumab emtansine achieved stable disease in some cases.[23] TKIs like gefitinib, erlotinib, and pan-HER inhibitors (lapatinib, neratinib) have been studied, with multitargeted TKIs (vandetanib, leflunomide) also showing promise by targeting HER and other pathways (e.g., platelet-derived growth factor receptor, vascular endothelial growth factor receptor).[24] Further phase III trials are needed to confirm these findings.

HER2-targeting antibodies like trastuzumab and pertuzumab show limited efficacy in HER2-mutated nonsmall cell lung cancer (NSCLC). Selective TKIs such as poziotinib and pyrotinib exhibit promising results, especially for HER2 exon 20 mutations, despite side effects.[25] T-DXd, an FDA-approved ADC, has shown significant antitumor activity in HER2-mutated NSCLC, with reduced adverse effects at lower doses. However, T-DM1 has limited efficacy in NSCLC patients, with only a few studies showing mild antitumor activity. Other ADCs, like RC48 and SHR-A1811, are under clinical trials for HER2-abnormal NSCLC. Further research is needed to improve outcomes.

In bladder cancer, HER2-targeting antibodies like trastuzumab, though not FDA-approved for bladder cancer, showed improved outcomes in combination with chemotherapy and radiotherapy but caused significant toxicity.[26] TKIs such as lapatinib, afatinib, and neratinib have shown promise, with afatinib improving progression-free survival in HER2/HER3-mutated cases.[27] ADCs like RC48, FDA-designated as "Breakthrough Therapy," have shown high ORR and survival benefits, especially in HER2-positive cases. T-DM1 and T-DXd also exhibit potential, though T-DXd combined with nivolumab has notable adverse effects. Further studies are needed to refine these treatments and manage toxicities.[28]

No HER2-targeted drugs are approved for biliary tract cancer (BTC), but trastuzumab showed a 66.6% ORR in HER2-amplified gallbladder cancer, and trastuzumab + pertuzumab achieved a 23% ORR in advanced BTC per the MyPathway trial.[29] Zanidatamab (ZW25) showed a 40% ORR, with further studies ongoing. Lapatinib lacked efficacy, while neratinib showed modest results.[30] HER2-targeting ADCs like RC48 and SYD985 showed promising outcomes, with ORRs of 36.4 and 25%, respectively.[31]

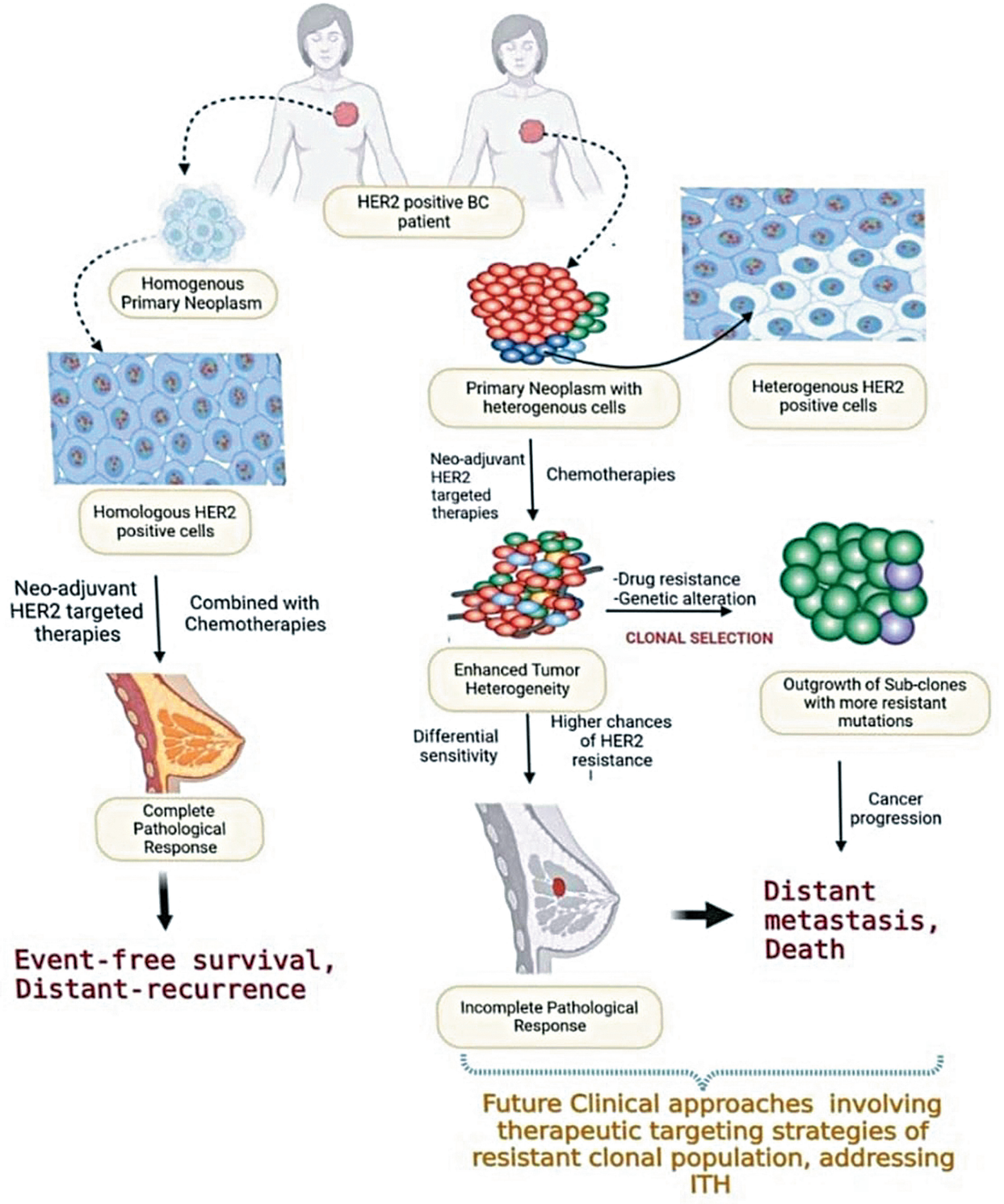

Fig. 2 Human epidermal growth factor receptor 2 (HER2) heterogeneity in breast cancer and its impact on therapy and disease progression.

HER2 monoclonal antibodies alone are ineffective in treating colorectal cancer, but combining them with TKIs improves outcomes. Trastuzumab and pertuzumab, combined with lapatinib, showed an ORR of 32% in metastatic colorectal cancer (mCRC).[32] Tucatinib and trastuzumab had an ORR of 55%, and pyrotinib and trastuzumab demonstrated promising results in ongoing trials. ADCs like T-DM1 and T-DXd also show efficacy. T-DM1 with pertuzumab offers a high disease control rate, and T-DXd monotherapy is recommended for HER2-amplified mCRC, but interstitial lung disease risk must be monitored.[33]

HER2 ITH, though rare in BC, is reported in significant percentages of other cancers, including gastric, gastro-esophageal junction, bladder, colorectal, lung, and

endometrial serous carcinoma. In GCs, HER2 ITH is observed in 14 to 79% by IHC and 23 to 54% by fluorescence in situ hybridization, higher than in BC. Endometrial serous carcinoma shows HER2 ITH in 31 to 53% of cases. However, no standard guidelines exist for evaluating HER2 heterogeneity in most nonbreast epithelial cancers, including GC, and its clinical significance remains unclear. Ongoing research focuses on novel and combination therapies to address HER2 variability, with ADCs showing promise so far.[34]

As the advantages of HER2-targeted therapies continue to expand, precise evaluation of HER2 status becomes increasingly critical, especially considering the variability in HER2 overexpression across different tumor types and within individual tumors. Alongside biological heterogeneity, technical issues such as the need for standardized and validated IHC and ISH protocols across histologies, the lack of specific guidelines for interpreting HER2 IHC in cancers beyond breast and gastric, and longer turnaround times due to growing workloads for technicians and pathologists present additional challenges. These factors emphasize the importance of further exploring HER2 expression in a broader range of cancers. Moreover, the demonstrated efficacy of ADCs in patients not traditionally considered HER2 "positive" highlights the necessity of gaining a deeper understanding of HER2 expression across all malignancies.[35] However, HER2-targeting drugs have shown promising antitumor effects in HER2-overexpressing non-BCs, but runing several well-designed clinical trials are warranted to prove potential positive efects of these drug in affected patients.[14,18,36]

Conclusion

HER2-targeted therapies have transformed cancer care, especially in more aggressive and advanced solid malignancies, offering hope for personalized treatment. However, challenges persist in refining diagnostic methods, ensuring equitable access, and balancing innovation with affordability. Well-designed clinical trials are essential to confirm these findings and optimize treatment regimens. While progress is ongoing, further data is needed to establish HER2-targeted therapies as a standard for nonbreast HER2-positive malignancies. Addressing these gaps is crucial to making precision medicine a reality for all.

Patient Consent

Patient consent is not required.

Conflict of Interest

None declared.

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