MEDICAL ONCOLOGY PATHOLOGY

Comprehensive Guide to HER2 Testing in Breast Cancer: Analyzing the ASCO-CAP 2023 Guideline Updates and Clinical Protocols

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The landscape of breast cancer diagnostics has undergone a transformative shift over the last two decades, primarily driven by the identification of the **Human Epidermal Growth Factor Receptor 2 (HER2)** as a critical biomarker. HER2, a product of the **ERBB2 gene**, is overexpressed or amplified in approximately 15% to 20% of all breast cancers, signifying a more aggressive clinical phenotype. However, the emergence of HER2-targeted therapies, such as trastuzumab, pertuzumab, and more recently, antibody-drug conjugates (ADCs) like trastuzumab deruxtecan (T-DXd), has revolutionized the prognosis for these patients. To ensure accurate patient selection for these potent therapies, the **American Society of Clinical Oncology (ASCO)** and the **College of American Pathologists (CAP)**provide rigorous guidelines for HER2 testing. The 2023 Guideline Update serves as a pivotal refinement of the standards set in 2018 and 2013, addressing evolving clinical needs and the nuance of the emerging 'HER2-low' terminology.

The Biological and Clinical Significance of HER2

HER2 is a transmembrane tyrosine kinase receptor belonging to the EGFR family. Unlike other members of this family, HER2 has no known ligand and exists in an active conformation, making it the preferred partner for heterodimerization with other EGFR receptors. When the **ERBB2 gene** is amplified, it leads to an overabundance of HER2 protein on the cell surface, triggering constitutive signaling through the **PI3K/AKT** and **MAPK**pathways. This results in uncontrolled cell proliferation, increased angiogenesis, and resistance to apoptosis.

Testing for HER2 status is mandatory for all newly diagnosed invasive breast cancers and all recurrent or metastatic lesions. The primary goal is to identify patients who will benefit from HER2-targeted agents. Historically, HER2 status was binary: positive or negative. However, the introduction of ADCs has necessitated a more granular understanding of HER2 expression levels, particularly in cases previously classified as 'negative' but exhibiting low-level protein expression.

Key Evolution: From 2018 to the 2023 Focused Update

The 2023 ASCO-CAP Guideline Focused Update was prompted by the results of the **DESTINY-Breast04 trial**, which demonstrated a significant survival benefit for patients with HER2-low (IHC 1+ or IHC 2+/ISH-negative) metastatic breast cancer when treated with trastuzumab deruxtecan. Despite the therapeutic implications, the 2023 update maintains a conservative approach to diagnostic terminology.

One of the most critical takeaways from the 2023 update is that there are **no current CAP or federal requirements**to use the term “HER2-Low” as a formal diagnostic category in pathology reports. Instead, the guideline reaffirms the importance of the existing scoring system (IHC 0, 1+, 2+, 3+) while emphasizing the need for precision in distinguishing IHC 0 from IHC 1+. This distinction is now clinically actionable, whereas it was previously considered irrelevant for therapeutic selection.

Technical Methodology: IHC and ISH Workflows

The evaluation of HER2 status relies on two primary methodologies: **Immunohistochemistry (IHC)**, which measures protein expression on the cell membrane, and **In Situ Hybridization (ISH)**, which measures the number of copies of the HER2 gene.

1. Immunohistochemistry (IHC) Scoring Rubric

IHC is typically the first-line test. The scoring depends on the intensity and the percentage of tumor cells showing complete membrane staining. The 2023 update confirms the 2018 scoring criteria but mandates high-power magnification (40x) for distinguishing between IHC 0 and 1+.

IHC Score	HER2 Status	Staining Pattern Criteria
0	Negative	No staining observed or membrane staining that is incomplete and is faint/ barely perceptible and within ≤ 10% of invasive tumor cells.
1+	Negative (Low)	Incomplete membrane staining that is faint/barely perceptible and within > 10% of invasive tumor cells.
2+	Equivocal	Weak to moderate complete membrane staining observed in > 10% of invasive tumor cells.
3+	Positive	Strong complete membrane staining observed in > 10% of invasive tumor cells.

2. In Situ Hybridization (ISH) Protocols

ISH is used as a reflex test for IHC 2+ (equivocal) cases. It utilizes fluorescent (FISH) or chromogenic (CISH) probes to count HER2 gene signals and **Chr17 (CEP17)** centromere signals. The determination is based on the **HER2/CEP17 ratio** and the **average HER2 copy number** per cell.

The 2018/2023 guidelines categorize ISH results into five groups to address complex patterns like co-amplification or polysomy of Chromosome 17:

- Group 1 (Positive): HER2/CEP17 ratio ≥ 2.0; Average HER2 copies ≥ 4.0.
- Group 2 (Positive with Reflex): HER2/CEP17 ratio ≥ 2.0; Average HER2 copies < 4.0. (Requires additional IHC review).
- Group 3 (Positive with Reflex): HER2/CEP17 ratio < 2.0; Average HER2 copies ≥ 6.0.
- Group 4 (Equivocal with Reflex): HER2/CEP17 ratio < 2.0; Average HER2 copies ≥ 4.0 and < 6.0.
- Group 5 (Negative): HER2/CEP17 ratio < 2.0; Average HER2 copies < 4.0.

Pre-Analytical Requirements and Quality Control

Technical accuracy in HER2 testing is highly sensitive to pre-analytical variables. Failure to adhere to these standards can result in false negatives (due to protein degradation) or false positives (due to edge artifacts).

Standardized Specimen Handling

1. Cold Ischemia Time: The time from tissue excision to the start of fixation must be less than 60 minutes.
2. Fixative: Only 10% neutral buffered formalin (NBF) should be used.
3. Fixation Duration: Specimens must be fixed for a minimum of 6 hours and a maximum of 72 hours.
4. Section Thickness: Sections should be cut at 4–5 μm for IHC to ensure consistent antibody penetration.

The Role of High-Power Magnification

The 2023 update places renewed emphasis on the use of the 40x objective lens. Pathologists must scrutinize 'negative' slides to detect faint, incomplete membrane staining. If any perceptible staining is found in more than 10% of the cells, the case must be upgraded from IHC 0 to IHC 1+. This shift is vital because IHC 0 patients may not currently qualify for certain ADCs, although research into the 'HER2-ultralow' (IHC >0 but <1+) category is ongoing.

The "HER2-Low" Paradigm: Clinical Implementation

While the 2023 ASCO-CAP update does not mandate the use of the label "HER2-Low," it acknowledges its clinical utility. Pathologists are encouraged to provide a **standardized pathology report**that clearly states the IHC score and the ISH results. The oncologist then uses these specific scores to determine eligibility for drugs like T-DXd.

Clinical Implications Matrix

IHC Score	ISH Result	Clinical Classification	Targeted Therapy Eligibility
3+	N/A	HER2-Positive	Standard HER2-targeted therapy (Trastuzumab, etc.)
2+	Positive	HER2-Positive	Standard HER2-targeted therapy
2+	Negative	HER2-Low	Eligible for T-DXd in metastatic settings
1+	N/A	HER2-Low	Eligible for T-DXd in metastatic settings
0	N/A	HER2-Negative	Generally ineligible for HER2-targeted therapy

Standardized Reporting and Reflex Testing Logic

A standardized report should include the specimen type (biopsy vs. resection), the site (primary vs. metastatic), and the specific antibody clone used (e.g., 4B5, HercepTest). When an initial test is equivocal (IHC 2+), reflex testing via ISH is mandatory. If the ISH result falls into Groups 2, 3, or 4, a second pathologist should review the IHC slides or the ISH signals to reach a final consensus.

The Importance of Testing Metastatic Sites

Research indicates that HER2 status can change during disease progression or in response to therapy (a phenomenon known as **receptor conversion**). The ASCO-CAP 2023 update reaffirms that at least one HER2 test should be performed on a metastatic site if available, as the status of the metastasis dictates the current treatment strategy more accurately than the primary tumor biopsy from years prior.

Case Study Analysis: Navigating Ambiguous Results

Case 1: The Borderline IHC 0/1+ Case. A core needle biopsy shows very faint, wispy staining in 15% of the tumor cells. Under 10x magnification, the slide appears negative. However, under 40x, the incomplete membrane staining is visible. Under the 2023 focus update, this should be reported as **IHC 1+**, allowing the patient access to ADC therapy in the metastatic setting.

Case 2: Polysomy 17 (Group 4 ISH). A sample returns an IHC score of 2+. ISH reveals an average HER2 copy number of 5.2 and a HER2/CEP17 ratio of 1.8. This is a Group 4 (Equivocal) result. The lab must perform a reflex IHC test on the same block or another block. If the reflex IHC is 3+, the case is positive. If the reflex IHC remains 2+ or less, the case is officially **HER2-Negative**, but the specific IHC score (1+ or 2+) must be noted for potential HER2-low treatment pathways.

Troubleshooting Common Pitfalls in HER2 Testing

- Heterogeneity: In some tumors, HER2 amplification occurs in clusters (focal amplification). If more than 10% of the cells show 3+ staining, the entire case is 3+. However, if the area is less than 10%, it should be reported as negative with a comment on the heterogeneous staining.

- Cytoplasmic Staining: IHC interpretation must only account for membranous staining. Granular cytoplasmic staining is a common artifact and should be ignored to avoid false positive IHC 2+ scores.
- Edge Artifact: Cells at the periphery of a tissue section often show non-specific, heavy staining. Pathologists must evaluate the center of the tissue to ensure the intensity is representative of the tumor's biology.
- Decalcification: Bone metastases often undergo decalcification, which can degrade DNA and protein. Using harsh acids for decalcification may lead to false negative HER2 results. Preferential use of EDTA is recommended for specimens requiring HER2 evaluation.

The Future of HER2 Testing: Quantitative Digital Pathology

The move toward 'HER2-low' and 'HER2-ultralow' definitions is pushing the limits of human visual perception through a microscope. The future of HER2 testing likely involves **Digital Pathology and Artificial Intelligence (AI)** . Image analysis algorithms can quantify the exact number of receptors per square micrometer and the precise intensity of DAB staining, providing a continuous rather than categorical scale of HER2 expression. This could eliminate inter-observer variability and provide a more reproducible metric for clinical trials.

Furthermore, the 2023 updates underscore the necessity of continuous education for laboratory personnel. Proficiency testing and internal validation of IHC assays are not just regulatory requirements but are essential components of patient safety. As new drugs target even lower levels of HER2 protein, the definition of 'negative' will continue to be scrutinized, potentially leading to a shift from binary classification to a nuanced, spectrum-based diagnostic model.

In conclusion, the 2023 ASCO-CAP HER2 testing guideline update serves as a critical bridge between established diagnostic standards and the new era of antibody-drug conjugates. By affirming the 2018 standards while emphasizing the clinical relevance of IHC 1+ and 2+ scores, the guidelines ensure that patients are neither over-treated with potentially toxic therapies nor denied life-extending targeted agents. Pathologists and oncologists must remain in close communication, ensuring that the nuances of the pathology report are fully translated into the clinical management plan. The ultimate goal remains unchanged: providing the right treatment to the right patient based on a foundation of rigorous, standardized, and technically sound biomarker testing.

Tags: #HER2 Testing #ASCO CAP Guidelines #Breast Cancer #Pathology Protocols #HER2 Low