Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

ASCO-CAP Guideline Update

Wolff, AC et al.

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1 Background & Methodology

Introduction

- ASCO and the CAP first published evidence-based clinical practice guidelines on HER2 testing in breast cancer in 2007,[1] and updated the guideline in 2013[2] and 2018.[3] This update assesses whether the 2018 recommendations remain valid.
- The impetus for revisiting the ASCO-CAP guideline was the 2022 publication of the DESTINY-Breast04 trial.
- Modi et al[4] showed in an open-label phase III study a significant improvement in survival in patients with breast cancers without HER2 overexpression or amplification, but with immunohistochemistry (IHC) 1+ or IHC 2+ with in-situ hybridization (ISH) not-amplified results, treated with the antibody-drug conjugate fam-trastuzumab-deruxtecan-nxki compared to physician's choice of chemotherapy after progression on other therapies for metastatic disease.
- Participants in the control arm did not have access to trastuzumab deruxtecan after progression and patients with IHC 0 results were excluded from the trial.

Introduction

- These data extended the FDA approved label indication of this drug and resulted in pre-market approval of the monoclonal IHC antibody testing system used in DESTINY-Breast04 (Ventana PATHWAY anti-HER2/neu 4B5 rabbit monoclonal antibody on the BenchMark ULTRA instrument) for a new use as a semiquantitative assay, to identify patients with breast cancer without HER2 overexpression or amplification who could be eligible for treatment with trastuzumab deruxtecan.
- The implications of these data for the ASCO-CAP breast cancer HER2 testing guideline recommendations were reviewed. The panel also provided commentary on testing reporting and best practices for identification and re.porting of IHC 0 vs IHC 1+ results.

Guideline Development Methodology

- The guideline process includes:
  - a systematic literature review by ASCO guidelines staff
  - an expert panel provides critical review and evidence interpretation to inform guideline recommendations
  - final guideline approval by ASCO EBMC and CAP Independent Review Panel

- The full ASCO Guideline methodology manual can be found at: www.asco.org/guideline-methodology

Clinical Questions

This clinical practice guideline addresses two overarching clinical questions:

1. What is the optimal testing algorithm for the assessment of HER2 status
2. What strategies can help ensure optimal performance, interpretation, and reporting of established assays?

Target Population and Audience

Target Population

- Patients with breast cancer.

Target Audience

- Medical oncologists, surgical oncologists, radiologists, pathologists, oncology nurses, patients, caregivers, advocates, and oncology advanced practice providers.

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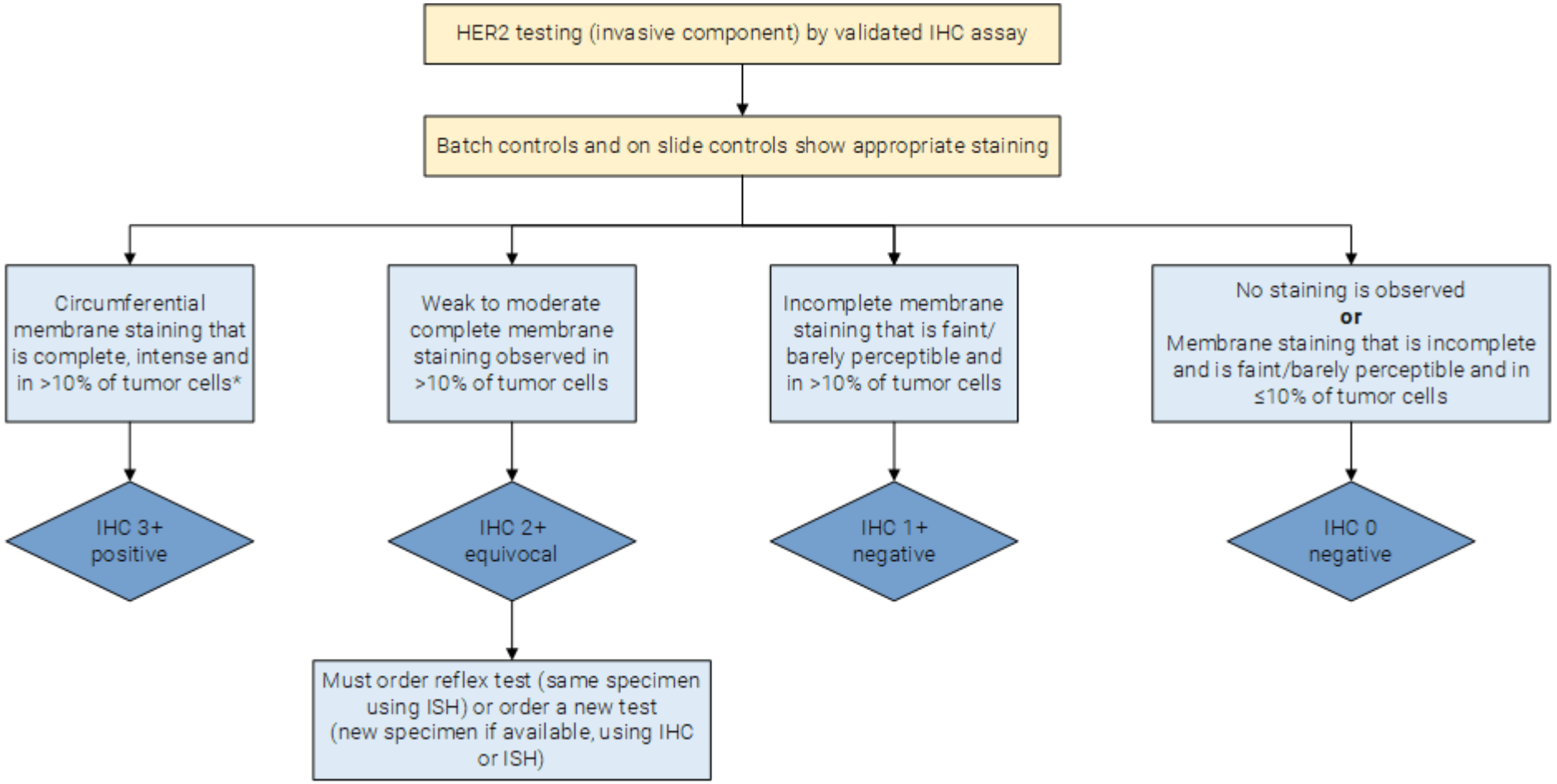
2 Recommendations

Recommendations

- The recommendations in previous (2013 and 2018) ASCO-CAP HER2 testing guideline updates are affirmed for classic anti-HER2 therapies that conventionally target HER2 signaling.
- While no changes are made to prior recommendations, there should be awareness that, for metastatic patients without HER2 over-expression or gene amplification, an IHC 1+ or 2+ result may make patients eligible for treatment targeting non-amplified/non-overexpressed levels of HER2 expression (and IHC 0 results would not), for which trastuzumab-deruxtecan is the only currently available agent. A new HER2 testing reporting footnote and best practices for identification and reporting of IHC 0 vs IHC 1+ results are offered in the discussion slides.
- See the end of the deck for a complete summary of unchanged recommendations.

Recommendations



NOTE. The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. Unusual staining patterns of HER2 by IHC can be encountered that are not covered by these definitions. In practice, these patterns are rare and if encountered should be considered IHC 2+ equivocal. As one example, some specific subtypes of breast cancers can show IHC staining that is moderate to intense but incomplete (basolateral or lateral) and can be found to be HER2 amplified. Another example is circumferential membrane IHC staining that is intense but within ≤10% of tumor cells (heterogeneous but very limited in extent). Such cases can be considered 2+ equivocal but additional samples may reveal different percentages of HER2 positive staining. (*)Readily appreciated using a low power objective and observed within a homogeneous and contiguous invasive cell population.

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3 Discussion

Discussion

- HER2 testing should still be optimized for the predictive purpose of identification of breast cancers with protein overexpression and/or gene amplification who could benefit from therapies aimed at disrupting HER2 signaling pathways.

Discussion

- While it is premature to change reporting terminology for lower levels of HER2 IHC expression (e.g., “HER2-Low”), pathology labs should include a footnote in their HER2 testing reports (IHC and ISH) with the following recommended comment: *“Patients with breast cancers that are HER2 IHC 3+ or IHC 2+/ISH amplified may be eligible for several therapies that disrupt HER2 signaling pathways. Invasive breast cancers that test “HER2-negative” (IHC 0, 1+ or 2+/ISH not-amplified) are more specifically considered “HER2-negative for protein overexpression/gene amplification” since non-overexpressed levels of the HER2 protein may be present in these cases. Patients with breast cancers that are HER2 IHC 1+ or IHC 2+/ISH not-amplified may be eligible for a treatment that targets non-amplified/non-overexpressed levels of HER2 expression for cytotoxic drug delivery (IHC 0 results do not result in eligibility currently).”*

Discussion

- HER2 IHC 1+ or 0 results are still both interpreted as “HER2-negative” (HER2 is not overexpressed) using the previously recommended scoring criteria. Importantly, the semi-quantitative IHC score must always be reported as well to ensure patients that meet eligibility criteria for trastuzumab deruxtecan can be identified.
  - Example: HER2-negative for protein over-expression (1+ staining present).

Discussion

- Since eligibility for trastuzumab deruxtecan (IHC 1+ or IHC 2+/ISH not-amplified) may hinge around the IHC 0/IHC 1+ threshold (even though the clinical validity of this threshold remains untested), pathologists can make best practice efforts to distinguish IHC 1+ results from 0 by the following practices:

    1. Examining HER2 IHC stained slides using standardized ASCO-CAP guidelines scoring criteria (see Figure 1 for interpretation).
    2. Examining HER2 IHC at high power (40x) when discriminating 0 from 1+ staining
    3. Considering second pathologist review when results are close to the 0 vs 1+ interpretive threshold (>10% of cells with incomplete membrane staining that is faint/barely perceptible).
    4. Using controls with a range of protein expression (including 1+) to help ensure the assay has an appropriate limit of detection.
    5. Careful attention to pre-analytic conditions of breast cancer tissue samples from both primary and metastatic sites.

Discussion

- Medical oncologists can also consider HER2 IHC results on prior or concurrent primary samples (or other metastatic sites) because there may be heterogeneity in HER2 expression levels between samples and metastatic cancer tissue samples may suffer from pre-analytic conditions that are not as well monitored as in primary breast tissue samples.

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4 Summary of Affirmed Recommendations

Summary of Affirmed Recommendations

Specimens to be Tested

- All newly diagnosed patients with breast cancer must have a HER2 test performed. Patients who then develop metastatic disease must have a HER2 test performed in a metastatic site, if tissue sample is available.

Summary of Affirmed Recommendations

Optimal Algorithm for HER2 Testing

- IHC 2+ (equivocal) is invasive breast cancer with weak to moderate complete membrane staining observed in >10% of tumor cells.

Evidence-based benefits outweigh harms	
Evidence Quality	**Strength of Recommendation**
High	Strong

- On the basis of some criteria (including a tumor grade 3), if the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen.

Evidence-based benefits outweigh harms	
Evidence Quality	**Strength of Recommendation**
High	Strong

Summary of Affirmed Recommendations

Optimal Algorithm for HER2 Testing

Evidence-based
benefits outweigh harms

Evidence Quality	Strength of Recommendation
Intermediate	Strong

- If a case has a HER2/CEP17 ratio ≥2.0 but the average HER2 signals/cell is <4.0, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/laboratory performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant assessment):
    a. If the IHC result is 3+, diagnosis is HER2 positive.
    b. If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasive cancer with IHC 2+ staining:
        i. If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category.
        ii. If the count remains an average of <4.0 *HER2* signals/cell and *HER2*/CEP17 ratio ≥2.0, the diagnosis is HER2 negative with a comment.[a]
    c. If the IHC result is 0 or 1+, diagnosis is HER2 negative with a comment. [a]

Summary of Affirmed Recommendations

Comment

[a] Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a *HER2*/CEP17 ratio ≥ 2.0 and an average *HER2* copy number of < 4.0 per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomly assigned to the trastuzumab arm did not seem to derive an improvement in disease-free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement ISH and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low *HER2* copy number by ISH and the lack of protein overexpression.

Summary of Affirmed Recommendations

Optimal Algorithm for HER2 Testing

Evidence-based benefits outweigh harms	
Evidence Quality	**Strength of Recommendation**
Intermediate	Strong

- If a case has an average of ≥6.0 *HER2* signals/cell with a *HER2*/CEP17 ratio of <2.0, formerly diagnosed as ISH positive for HER2, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/lab performing the ISH test, IHC testing and the slides from both ISH and IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant review):
    a. If the IHC result is 3+, diagnosis is HER2 positive.
    b. If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasion with IHC 2+ staining:
        i. If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category.
        ii. If the *HER2*/CEP17 ratio remains <2.0 with ≥6.0 *HER2* signals/cell, the diagnosis is HER2 positive.
    c. If the IHC result is 0 or 1+, diagnosis is HER2 negative with comment.[b]

Summary of Affirmed Recommendations

Comment

[b] There are insufficient data on the efficacy of HER2-targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative.

Summary of Affirmed Recommendations

Optimal Algorithm for HER2 Testing

Evidence-based
benefits outweigh harms

Evidence Quality	Strength of Recommendation
Intermediate	Strong

- If the case has an average *HER2* signals/tumor cell of ≥4.0 and <6.0 and the *HER2*/CEP17 ratio is <2.0, formerly diagnosed as ISH equivocal for HER2, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/laboratory performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant review):
    a. If the IHC result is 3+, diagnosis is HER2 positive.
    b. If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasion with IHC 2+ staining:
        i. If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category.
        ii. If the count remains an average of ≥4.0 and <6.0 *HER2* signals/cell with *HER2*/CEP17 ratio <2.0, the diagnosis is HER2 negative with a comment.[c]
    c. If the IHC result is 0 or 1+, diagnosis is HER2 negative with a comment.[c]

Summary of Affirmed Recommendations

Comment

[c] It is uncertain whether patients with an average of ≥ 4.0 and < 6.0 *HER2* signals per cell and a *HER2*/CEP17 ratio of < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH ratio threshold for positive, there is a higher likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen

Summary of Affirmed Recommendations

ISH Rejection Criteria

- Test is rejected and repeated if:
  - Controls are not as expected
  - Observer cannot find and count at least two areas of invasive tumor
  - > 25% of signals are unscorable due to weak signals
  - > 10% of signals occur over cytoplasm
  - Nuclear resolution is poor
  - Autofluorescence is strong
- Report HER2 test result as Indeterminate as per parameters described.

Summary of Affirmed Recommendations

ISH Interpretation

- The pathologist should scan the entire ISH slide prior to counting at least 20 cells or use IHC to define the areas of potential *HER2* amplification.
- If there is a second population of contiguous cells with increased *HER2* signals/cell and this cell population consists of > 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or IHC slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and reported.

Acceptable (IHC and ISH) Tests

- Should preferentially use an FDA-approved IHC, brightfield ISH, or FISH assay.

Summary of Affirmed Recommendations

IHC Rejection Criteria

- Test is rejected and repeated or tested by FISH if:
  - Controls are not as expected
  - Artifacts involve most of sample
- Sample has strong membrane staining of normal breast ducts (internal controls)

IHC Interpretation Criteria

- Should interpret IHC test using a threshold of more than 10% of tumor cells that must show homogeneous, dark circumferential (chicken wire) pattern to call result 3+, HER2 positive.

Summary of Affirmed Recommendations

Reporting Requirements for All Assay Types

- Report must include guideline-detailed elements except for changes to reporting requirement and algorithms defined in these recommendations.

Optimal Tissue Handling Requirements

- Time from tissue acquisition to fixation should be as short as possible; samples for HER2 testing are fixed in 10% neutral buffered formalin for 6-72 hours; cytology specimens must be fixed in formalin.
- Samples should be sliced at 5- to 10-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of neutral buffered formalin.
- Any exceptions to this process must be included in report.

Summary of Affirmed Recommendations

Optimal Tissue Sectioning Requirements

- Sections should ideally not be used for HER2 testing if cut > 6 weeks earlier; this may vary with primary fixation or storage conditions.

Optimal Internal Validation Procedure

- Validation of test must be performed before test is offered.

Summary of Affirmed Recommendations

Optimal Initial Test Validation

- Laboratories performing these tests should be following all accreditation requirements, one of which is initial testing validation. The laboratory should ensure that initial validation conforms to the published 2010 ASCO-CAP Recommendations for IHC Testing of ER and PgR guideline validation requirements with 20 negative and 20 positive for FDA-approved assays and 40 negative and 40 positive for LDTs. This requirement does not apply to assays that were previously validated in conformance with the 2007 ASCO-CAP HER2 testing guideline, and who are routinely participating in external proficiency testing for HER2 tests, such as the program offered by the CAP.
- Laboratories are responsible for ensuring the reliability and accuracy of their testing results, by compliance with accreditation and proficiency testing requirements for HER2 testing assays. Specific concordance requirements are not required.

Summary of Affirmed Recommendations

Optimal Monitoring of Test Concordance Between Methods

- See text under “Optimal Laboratory Accreditation”.

Optimal Internal QA Procedures

- Should review and document external and internal controls with each test and each batch of tests.
  - Ongoing quality control and equipment maintenance
  - Initial and ongoing laboratory personnel training and competency assessment
  - Use of standardized operating procedures including routine use of control materials
  - Revalidation of procedure if changed
  - Should perform ongoing competency assessment and document the actions taken as a part of the laboratory record.

Summary of Affirmed Recommendations

Optimal External Proficiency Assessment

- Participation in and successful completion of external proficiency testing program with at least two testing events (mailings) a year
  - Satisfactory performance requires at least 90% correct responses on graded challenges for either test
  - Unsatisfactory performance will require laboratory to respond according to accreditation agency program requirements

Optimal Laboratory Accreditation

- Onsite inspection every other year with annual requirement for self-inspection
  - Reviews laboratory validation, procedures, QA results and processes, results and reports
  - Unsatisfactory performance results in suspension of laboratory testing for HER2 for that method

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5 Additional Information

Additional Resources

- More information, including a supplement and clinical tools and resources, is available at www.asco.org/breast-cancer-guidelines

- Patient information is available at www.cancer.net

Update Expert Panel Members

Name	Affiliation/Institution	Role/Area of Expertise
Kimberly H. Allison, MD (Co-Chair)	Stanford University School of Medicine, Stanford, CA	Pathology
Antonio Wolff, MD (Co-Chair)	Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD	Medical Oncology
Michell Dowsett, PhD	The Royal Marsden NHS Foundation Trust, London, United Kingdom	Molecular Pathology
M. Elizabeth H. Hammond, MD	Intermountain Healthcare and University of Utah School of Medicine, Salt Lake City, UT	Pathology
Daniel F. Hayes, MD	University of Michigan, Ann Arbor, MI	Medical Oncology
Lisa M. McShane, PhD	National Cancer Institute, Bethesda, MD	Biostatistics
Thomas J. Saphner, MD	Vince Lombardi Cancer Clinic, Two Rivers, WI	Community Oncology
Patricia A. Spears, BS	University of North Carolina, Chapel Hill, NC	Patient Advocacy
Mark R. Somerfield, PhD	American Society of Clinical Oncology (ASCO), Alexandria, VA	ASCO Practice Guideline Staff (Health Research Methods)

Abbreviations

- ASCO, American Society of Clinical Oncology
- CAP, College of American Pathologists
- CEP17, chromosome enumeration probe 17
- EBMC, Evidence Based Medicine Committee
- ER, estrogen receptor
- FDA, US Food and Drug Administration
- FISH, fluorescent in situ hybridization
- HER2, human epidermal growth factor receptor 2
- IHC, immunohistochemistry
- ISH, in situ hybridization
- LDT, laboratory-developed test
- PgR, progesterone receptor
- QA, quality assurance

References

1. 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(1):18-43.
2. 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. *Arch Pathol Lab Med*. 2014; 138(2):241-256.
3. Wolff AC, Hammond EH, 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. *Arch Pathol Lab Med*. 2018;142(11):1364-1382.
4. Wolff AC, Somerfield MR, Dowsett M, et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO-CAP guideline update [published online June 7, 2023]. *Arch Pathol Lab Med.* 2023. doi:10.5858/arpa.2023-0950-SA
5. Modi S, Jacot W, Yamashita T, et al: Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med 387:9-20, 2022

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