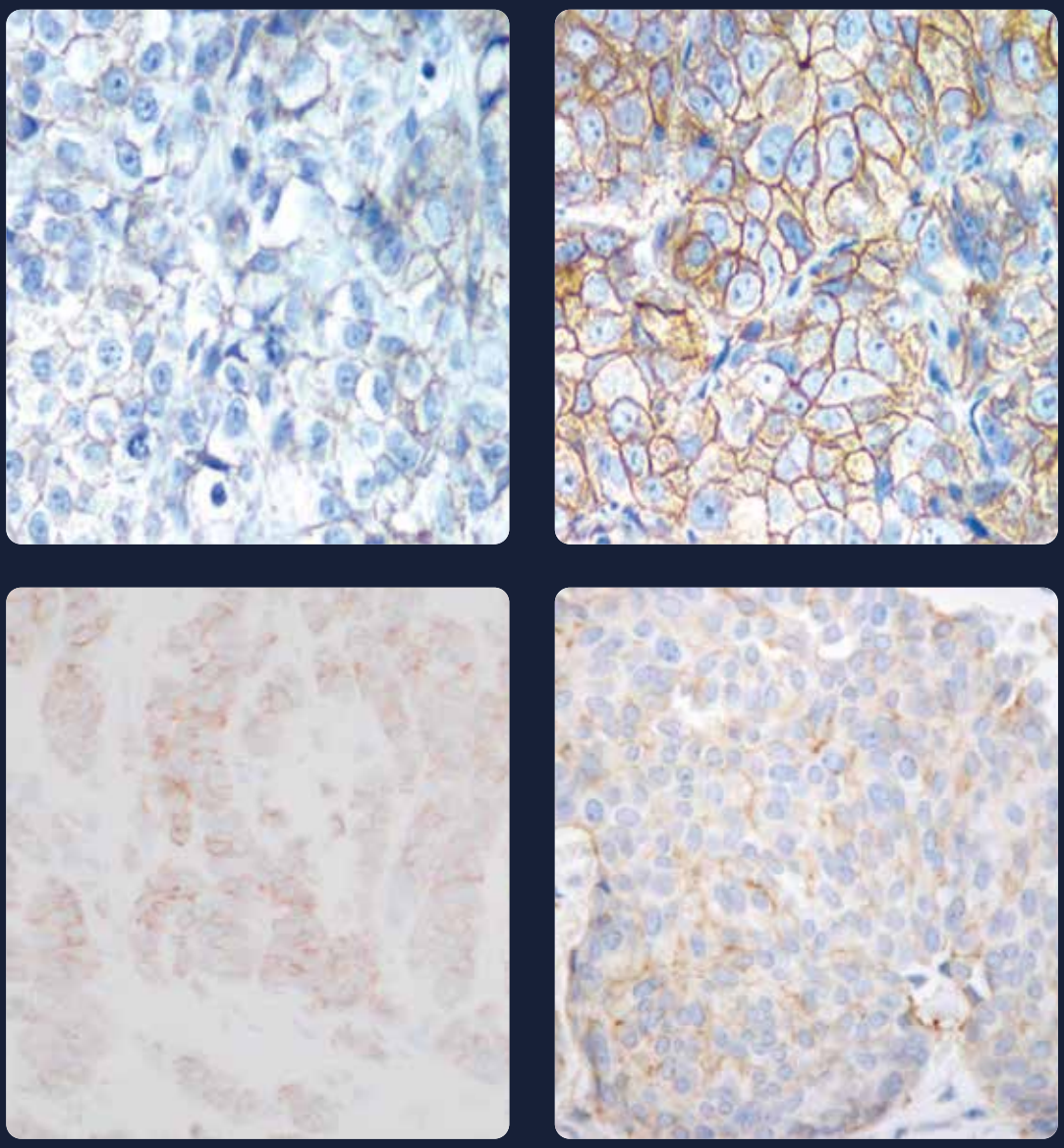
HER2 expression in breast cancer

A comprehensive reference for IHC analysis



This educational resource has been developed in collaboration with pathologists who specialize in HER2 IHC evaluation in breast cancer.

HER2 Know.com, a peer-led educational platform by pathologists, for pathologists

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

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Daiichi-Sankyo | AstraZeneca

Contents

Introduction

Intended use

This comprehensive reference was developed in partnership with a faculty of breast pathologists, as a peer-led educational resource to help support and maintain good clinical practice when analyzing HER2 staining patterns.

This reference book provides useful information for approaching HER2 IHC scoring across the full range of HER2 expression.

TIPS FOR QR CODE SCANNING

1. Focus your smartphone camera on the QR code so all four edges of the QR code are on the phone's screen.

2. When the code is detected, tap on the notification that appears to view the QR code content on HER2Know.com.

Illustrative cases

Illustrative case examples depict breast carcinomas stained with HER2 IHC antibodies. Images and case descriptions are provided solely for educational purposes.

QR code

The associated QR codes are linked directly to the digital image of the presented case on HER2Know.com.

Additional information, educational resources, and an extensive HER2 IHC image gallery can be found at **HER2**Know.com

Clinical background

Significance of HER2

Human epidermal growth factor receptor 2 (HER2) gene (also referred to as *ERBB2*) is amplified and/or overexpressed in approximately 15% to 20% of primary breast cancers.[1] The relationship between breast cancer HER2 expression and prognosis was recognized in the 1980s and HER2 has since proven to be a significant prognostic and predictive biomarker in breast cancer.[2]

Overexpression of HER2 is linked to aggressive histological characteristics, a poor prognosis, and reduced overall survival, but it also predicts response to HER2-directed therapies.[3,4] Breast cancers are classified by hormone receptor (HR) status (positive or negative; HR+/-) and HER2 status (positive or negative; HER2+/-) (**Figure 1**). These distinct molecular subtypes give important prognostic information and help guide treatment decisions.[5]

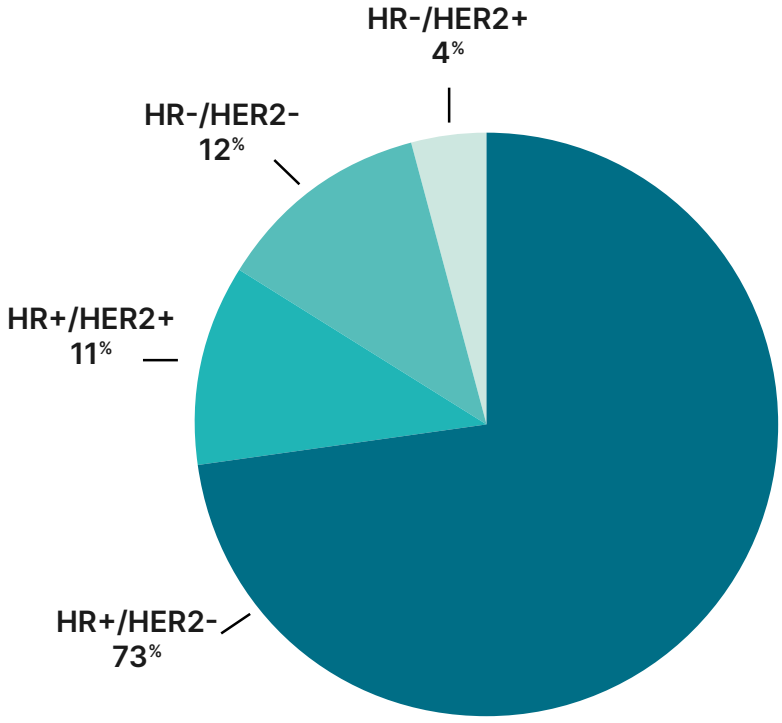


Figure 1: Estimated incidence of female breast cancer molecular subtypes (US)[4]

	Luminal A	Luminal B (HER2-)	Luminal B (HER2+)	HER2 enriched/ overexpression	TNBC
ER	+	+	+	–	–
PR	High	Low*	Any	–	–
HER2	-	-	+	+	-
Ki67	Low	High*	Any		

Adapted from Senkus et al. 2015.[5]

HER2-DIRECTED THERAPIES

Early clinical studies with the first FDA-approved HER2-directed agent marked a significant clinical revolution in the management of patients with HER2-positive disease.[6,7]

The therapeutic landscape has since continued to evolve with the development of several HER2 targeting agents that have improved prognosis in HER2-positive breast cancer.[8]

*Either PR low or Ki67 high.
ER, estrogen receptor; FDA, U.S. Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; Ki-67, proliferation marker; PR, progesterone receptor; TNBC, triple negative breast cancer.
1. Wolff AC, et al. J Clin Oncol 2013;31:3997-4013. 2. Slamon DJ, et al. Science 1989;244(4905):707-712. 3. Marchiò C, et al. Semin Cancer Biol 2020;72:123-135. 4. American Cancer Society, Inc., Surveillance Research Breast Cancer Facts & Figures 2019-2020. 5. Senkus E, et al. Ann Oncol 2015;26(Suppl 5):v8-v30. 6. Pernas S and Tolaney S. Ther Adv Med Oncol 2019;11:1-16. 7. Dieci M, et al. Cancer Treat Rev 2020;88. 8. Tarantino P, et al. J Clin Oncol 2020;38(17):1951-1962.

HER2 assessment

Validated techniques

HER2 status should be assessed in all newly diagnosed patients with invasive primary breast carcinomas and in recurrent and metastatic tumors whenever biopsy tissue is available.[1]

The common and standard methods for determining HER2 status in breast cancer are immunohistochemistry (IHC) and *in situ* hybridization (ISH). IHC analysis determines the level of HER2 protein expression and ISH, a cytogenetic technique, detects amplification of the HER2 *(ERBB2)* gene.[1,2] (**Figure 2**)

Both methods use formalin-fixed, paraffin-embedded (FFPE) tissue samples[2] and may be considered complementary in nature.[3]

Several FDA-approved HER2 IHC and ISH assays are commercially available to aid assessment of HER2. Distinct applications of HER2 ISH techniques include fluorescent ISH (FISH), chromogenic ISH (CISH), interphase quantitative fluorescent ISH (IQFISH), silver-enhanced ISH (SISH), and bright-field double ISH (BDISH), all of which target host DNA.[2]

Other emerging methods for assessing HER2 (not currently guideline-recommended) include RNA-based methodologies such as quantitative reverse transcription polymerase chain reaction (qRT-PCR)[4] and targeted mass spectrometry using FFPE tissue.[5] HER2 gene amplification can also be detected using Next Generation Sequencing (NGS).[6]

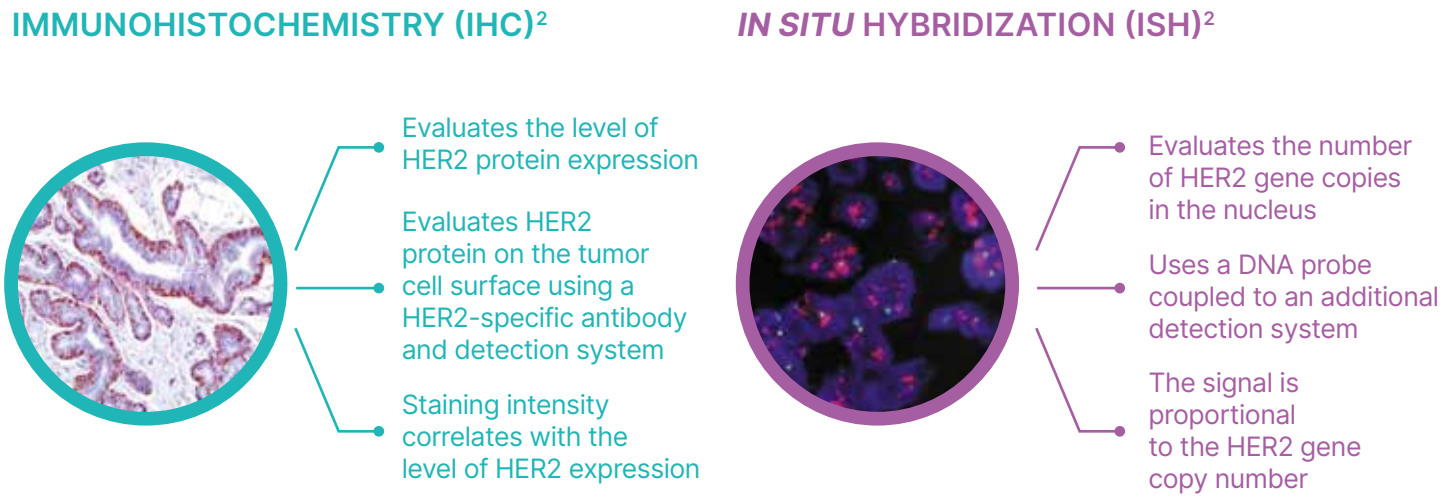


Figure 2: Standard techniques for assessing HER2 status[2]

BDISH, bright-field double *in situ* hybridization; CISH, chromogenic *in situ* hybridization; FDA, U.S. Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization; IQFISH, interphase quantitative fluorescent *in situ* hybridization; NGS, next generation sequencing; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SISH, silver-enhanced *in situ* hybridization.

1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 2. Furrer D, et al. Am J Clin Pathol 2015;144:686-703. 3. Hicks DG and Schiffhauer L. Lab Medicine 2011;42:459-467. 4. Dabbs DJ, et al. J Clin Oncol 2011;29(32):4279-4285. 5. Hembrough T, et al. J Mol Diagn 2013;15:454-465. 6. Nakamura K, et al. Med Oncol. 2021;38(4):36.

Sample considerations

Tissue from the primary tumor can be obtained through a core needle biopsy (CNB), as well as from an incisional and excisional surgical procedure.[1] If the initial HER2 test result in a CNB specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen based on specific clinical criteria[2]:

- Tumor is grade 3
- Amount of invasive tumor in the core biopsy specimen is small
- Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core
- Core biopsy result is equivocal for HER2 after testing by both ISH and IHC
- There is doubt about the handling of the core biopsy specimen (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

Patients who develop metastatic disease must have a HER2 test performed at metastatic site, if tissue sample is available.[2]

Common sites of distant metastases include bone (48%), liver (27%), lung (23%), central nervous system (CNS) (17%), and pleura (7%) *(of 531 patients with breast cancer associated metastases between 1997 and 2010).*[3]

There are several key considerations for using CNB, including consistency of sample fixation, heterogeneity, and artifacts.[1,4]

Sample fixation

Tissue samples from CNB are usually placed in formalin in a timelier fashion compared with excisional biopsies.[5] This ensures that cold ischemic time can be as short as possible, ideally <1 hour.[1] Formalin is also likely to infiltrate more quickly (because of the smaller size), resulting in a more consistent tissue fixation.[2,5]

Artifacts

Crush and edge artifacts particularly affect core biopsies and may hinder interpretation of staining.[5] Tissue samples with staining artifacts should be interpreted with caution to avoid over-interpretation of aberrant staining.[5]

Heterogeneity

Breast tumors that exhibit heterogeneity may poorly reflect histological features and biological profile of the entire tumor. Repeat testing should be considered if results appear discordant with histopathologic findings.[4,5]

A disclaimer is required on HER2 testing report for decalcified tissue, unless the assay has been previously validated for decalcified tissues.[5]

CNB, core needle biopsy; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.

1. Wolff AC, et al. J Clin Oncol 2013;31:3997-4013. 2, Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 3. Soni A, et al. Am J Clin Pathol 2015;143(4):471-478. 4. Wolff A. J Clin Oncol 2007;25(1): 118-145. 5. Hicks DG and Schiffhauer L. Lab Medicine 2011;42:459-467.

ASCO-CAP guidelines for HER2 testing in breast cancer

The pathologist's critical evaluation of HER2 has an important role in determining the diagnosis, prognosis, and appropriate treatment options. Since 2007, the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have provided guidance and recommendations for HER2 testing.[1] These clinical practice guidelines are periodically updated to meet changing practice needs and incorporate new evidence.[1]

The 2018 ASCO-CAP HER2 testing guidelines include algorithms for the interpretation of results from IHC and ISH assays.[1] (Figure 3)

The 2023 ASCO-CAP guideline update affirms the 2018 ASCO-CAP guideline recommendations on HER2 testing in breast cancer and offers additional guidance around reporting HER2 IHC 1+, IHC 2+/ISH- results, and best practices for differentiating IHC 1+ and 0 tumors.[2]

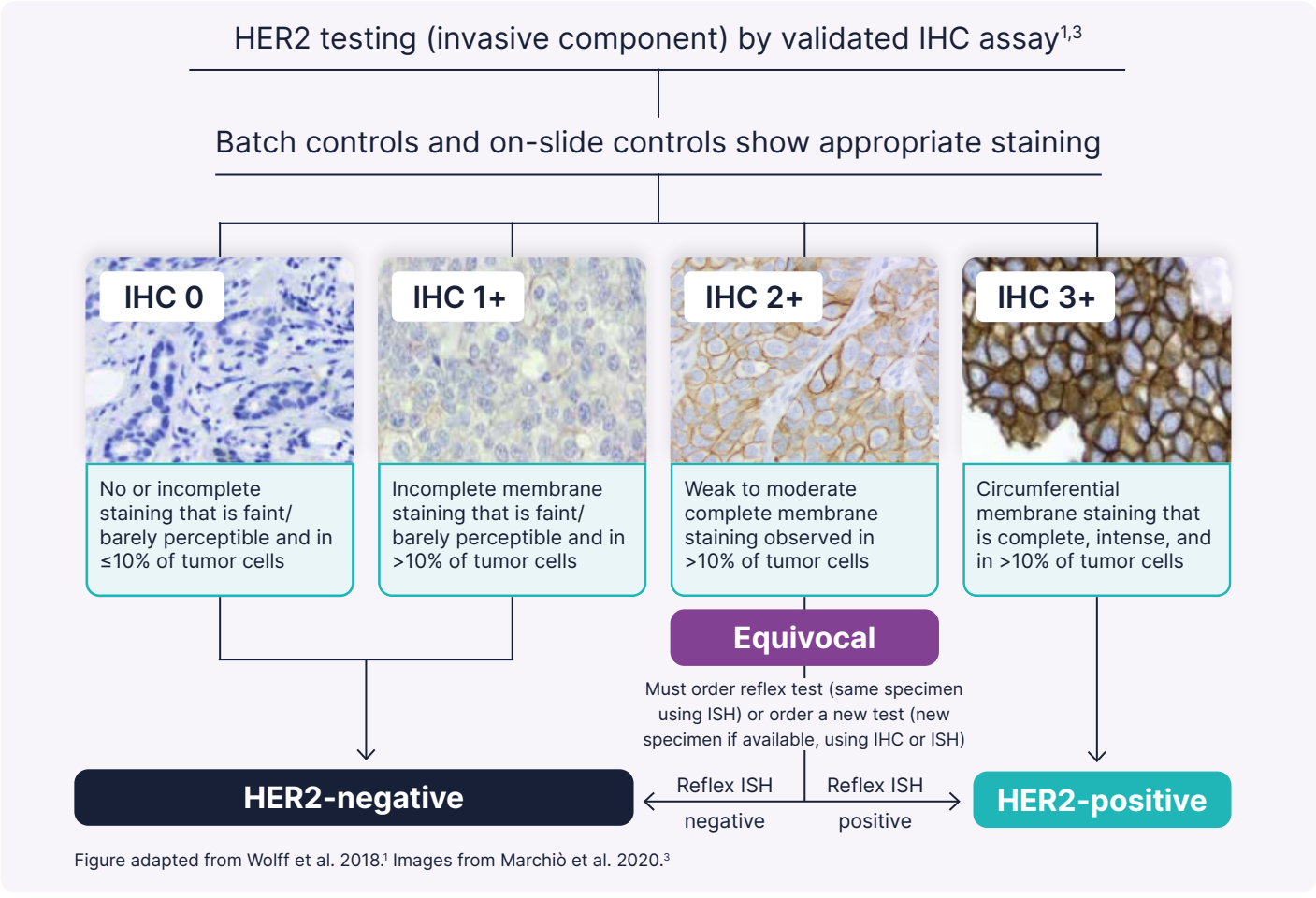


Figure adapted from Wolff et al. 2018.[1] Images from Marchiò et al. 2020.[3]

Figure 3: 2018 ASCO-CAP guidelines HER2 testing algorithm[1,3]

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.

1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 2. Wolff AC, et al. Arch Pathol Lab Med 2023. https://doi.org/10.5858/arpa.2023-0950-SA. 3. Marchiò C, et al. Semin Cancer Biol 2020;72:123-135.

Variables affecting HER2 IHC results

Variability in clinical interpretation of HER2 results can be minimized through standardization of testing processes to improve consistency in diagnostic assessment for HER2 status.[1-3]

Pre-analytic

Reproducibility of HER2 interpretation is also influenced by the following pre-analytical variable requirements:

RECOMMENDATION	RATIONALE
Cold ischemic time should be as short as possible (ideally <1 hour)[4]	Prompt fixation preserves sample quality; fixation delays compromise sample integrity and can adversely affect HER2 IHC interpretation[3]
Fixation should be fixed in 10% neutral buffered formalin (NBF) - clinically validated for HER2[1]	Alternative fixative methods not specified by individual assay manufacturers may be considered off-label[3]
Fixation time should be no less than 6 hours and no more than 72 hours before processing[1]	Under- or over-fixation of tissue may increase the likelihood of false negative and false positive results[3]
Samples should be sliced at 5-10 mm intervals[1]	Allows for formalin infiltration and uniform chemical fixation throughout the tissue[3]
Sections should be cut within 6 weeks of HER2 staining[1]	Ensures antigenicity[5]

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NBF, neutral buffered formalin.
1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 2. Tarantino P, et al. J Clin Oncol 2020;38(17):1951-1962. 3. Hicks DG and Schiffhauer L. Lab Medicine 2011;42:459-467. 4. Wolff AC, et al. J Clin Oncol 2013;31:3997-4013. 5. Wolff A. J Clin Oncol 2007;25(1): 118-145.

Variables affecting HER2 test results

Analytic

The use of FDA-approved IHC and ISH test kits containing standardized, high-quality reagents of known specificity and sensitivity are recommended to help ensure reproducible and consistent results.[1]

HER2 IHC tests need to be validated before clinical use.[1] Validation is performed by testing 25-100 samples that have been fixed in formalin using the standardized operating procedure for the laboratory, and then tested in parallel by an alternative method (ISH if validating IHC) using a previously validated assay in either the same laboratory or in another laboratory.[2] Tissue controls should be run with every assay to help ensure proper assay performance.[1]

Interpretation criteria

The 2018 ASCO-CAP guideline recommendations provide guidance for standardizing HER2 IHC interpretation, and these include defined threshold criteria for the pattern/intensity of membrane staining, as well as for proportion of stained tumor cells.[1] Interpretation may slightly vary based on interpretation guides for specific IHC assays.

Quality assurance

Laboratories performing HER2 IHC should follow all accreditation requirements including initial test validation, optimal internal quality assurance procedures, and optimal external proficiency assessment to maintain laboratory accreditation. Adherence to proficiency and accreditation requirements improves standardization of results.[1,3]

Reporting elements

CAP encourage laboratories performing HER2 tests to provide reports in synoptic format which ensures all relevant information is captured.[4] Distinct IHC score matched to HER2 clinical status, staining intensity, test type, and primary antibody are included as core data elements to be reported.[4] In the event of IHC 2+ cases, a statement is required to indicate supplementary reflex ISH test should be included.[4]

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FDA, U.S. Food and Drug Administration; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.

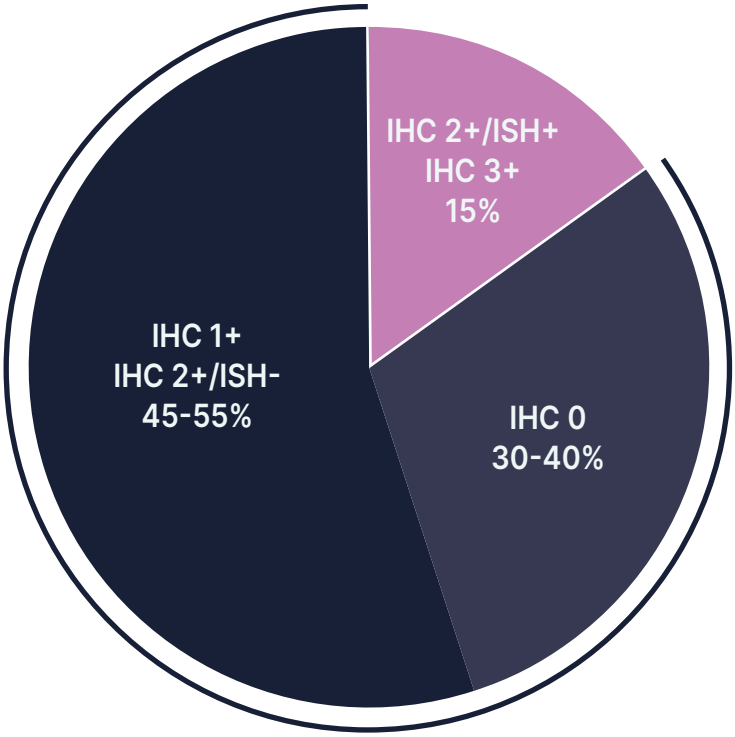
1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 2. Hicks DG and Schiffhauer L. Lab Medicine 2011;42:459-467. 3. CAP proficiency testing (PT)/external quality assessment (EQA). 4. Fitzgibbons PL, et al. Template for Reporting Results of Biomarker Testing of Specimens from Patients with Carcinoma of the Breast.

The HER2 IHC spectrum

The category of HER2 expressing breast cancer involves a spectrum of carcinomas with different degrees of HER2 expression.[1] However, based on the 2018 ASCO-CAP HER2 testing guidelines, clinical classification of HER2 status is binary and optimized to identify breast cancer patients with HER2-positive breast tumors.[2]

Breast tumors are considered HER2-positive when there is evidence of HER2 overexpression by IHC assay (score IHC 3+).[2] Additionally, tumors that are IHC 2+ and have gene amplification by ISH assay on at least one sample are also classified as HER2-positive.[2]

For cases that are IHC 0 and 1+, or IHC 2+ with a negative ISH assay, the tumor is considered HER2-negative. The exception are dual-probe ISH testing group 3 results HER2/CEP17 ratio <2.0; average HER2 copy number ≥6.0 signals per cell where tumors that are IHC 2+ are deemed HER2-positive.[3]



Approximately 85% of breast tumors are classified as HER2-negative.

Figure 4: Percentage of breast cancers with various levels of HER2 expression[3]

Prevalence of HER2-low breast cancer

Approximately 45-55% of breast cancers could be classed as HER2-low (**Figure 4**).[3] These patients are currently classified as TNBC or luminal BC (if HRs are expressed).[3]
Several clinical studies have described tumors with low levels of expression as having a HER2 IHC assay score of 1+ or 2+ without gene amplification (ISH negative).[3,4]

With growing clinical evidence for tumors with low levels of HER2 expression, it is becoming increasingly important to differentiate HER2 IHC 1+ and 2+ from HER2 IHC 0 in a reproducible manner.[1,3]

ASCO, American Society of Clinical Oncology; BC, breast cancer; CAP, College of American Pathologists; CEP17, chromosome enumeration probe 17; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; ISH, *in situ* hybridization; TNBC, triple negative breast cancer.

1. Marchiò C, et al. Semin Cancer Biol 2020;72:123-135. 2. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 3. Tarantino P, et al. J Clin Oncol 2020;38(17):1951-1962. 4. Agostinetto, E. et al. Cancers 2021;13:2824.

How to approach scoring

General IHC workflow process

Critical evaluation of a HER2 IHC assay should begin with establishing an evaluable specimen. This includes a review of the batch controls and the on-slide positive control. The slide containing the patient's tissue sample should be scanned to help ensure an invasive tumor is easily identifiable and no significant HER2 staining is present within normal breast epithelial cells. When the controls are assessed and the normal breast elements are negative, then the invasive component of the patient's breast tumor sample can be further evaluated for HER2 expression.[1]

Recommendation[1]	Rationale[1]
Run positive and negative controls with every assay	Ensures proper assay performance and calibration of assay sensitivity and dynamic range
Include a sample of tumor known to be HER2-positive on the same slide as the test sample	Ensures all reagents are dispensed onto the slide

Evaluating staining pattern and intensity

Intense staining - readily visible 'chicken-wire' pattern at **4X(5X)***[2,3]

Examine the entire breast tissue section to identify HER2 sections that should be scored and estimated for percentage of tumor cells with membrane staining.[4] The pattern and intensity of staining suggestive of strongly positive cases will be visible at 4X(5X)* magnification.[5]

Moderate staining - visible at **4X(5X)***, confirmed at **10-20X**[2,3]

If resolving equivocal IHC 1+/2+ cases with 10X magnification is difficult, confirm score with 20X or 40X.[5]

Weak staining - hardly perceptible at **4X(5X)*** and **10X**, visible at **20X** and confirmed at **40X**[2,3]

Examine these cases at 40X or higher magnification to discriminate between IHC 0 and 1+.[4]

*Depending on the available type of magnifiers, pathologists will use 4X or 5X.

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry.

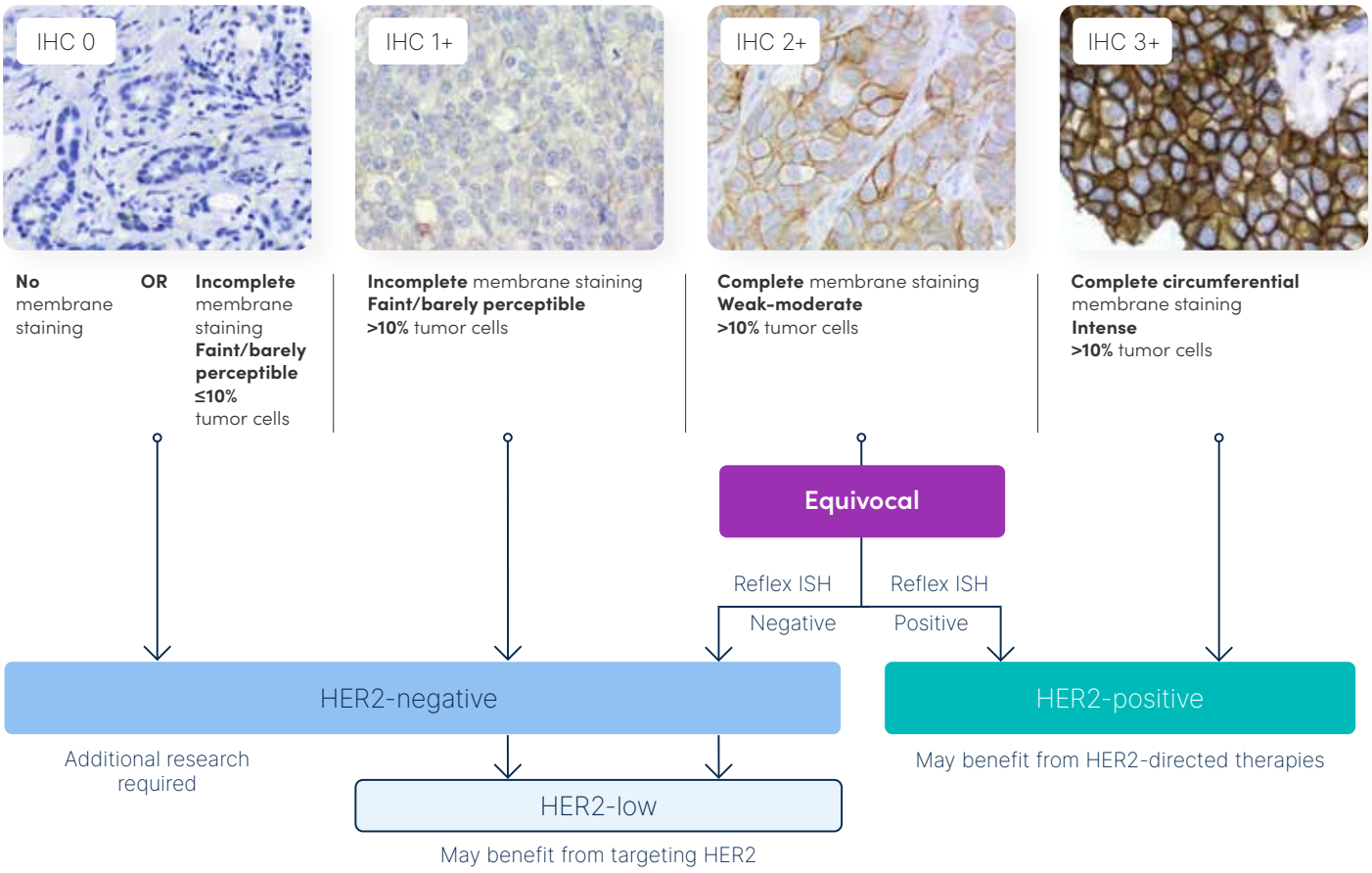
1. Hicks DG and Schiffhauer L. Lab Medicine 2011;42:459-467. 2. AstraZeneca and Daiichi Sankyo. Data on file. REF-18157. 2022. 3. Franchet C et al. Annales de Pathologie 2021;41(6):507-520. 4.Interpretation Guide PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody Staining of Breast Carcinoma, 1499100 Rev K, 2019-07-15. 5. DAKO HercepTest TM Interpretation Manual Breast Cancer 28630 17SEP14 - ROW Version.

How to approach scoring

HER2 TESTING ALGORITHM

Breast tumors with HER2 IHC 1+ or IHC 2+/ISH- are currently considered HER2-negative. Recently, alternative algorithms have been proposed to reflect the complete range of HER2 expression, incorporating a category for low levels of HER2 expression.[1] (**Figure 5**)

Proposal of an algorithm for defining breast cancer with low levels of HER2 expression



Images adapted from Marchiò C, et al. 2020. Figure adapted from Wolff AC, et al. 2018.[1,2]

Figure 5: Proposed algorithm for HER2 testing inclusion of HER2-low[2]

HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.
1. Marchiò C, et al. Semin Cancer Biol 2020;72:123-135. 2. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122.

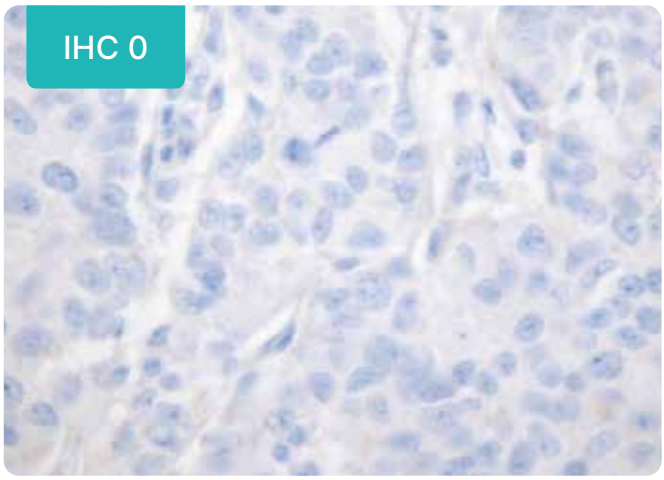
Interpretation of HER2 staining patterns

Scoring guidelines

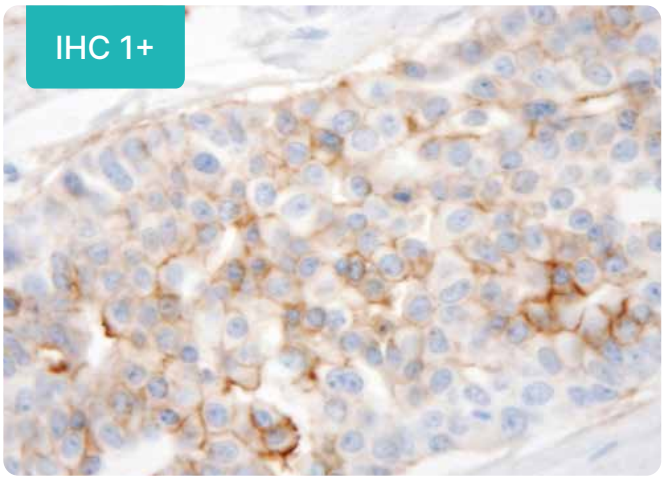
The 2018 ASCO-CAP guidelines classify breast cancer as[1]:

HER2-negative:
IHC 0, IHC 1+, or IHC 2+/ISH-

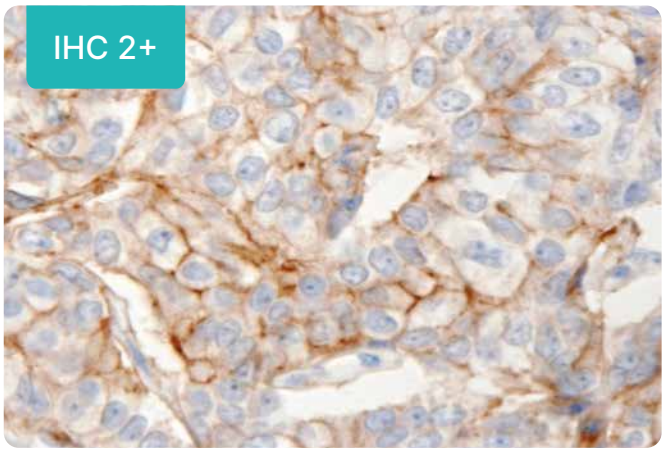
HER2-positive:
IHC 2+/ISH+, or IHC 3+



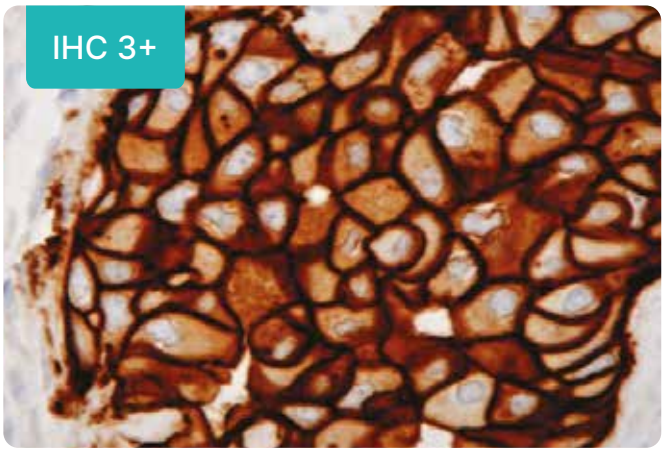
No staining is observed or membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells.



Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells.



Weak to moderate complete membrane staining observed in >10% of tumor cells.



Circumferential membrane staining that is complete, intense, and in >10% of tumor cells.

Figure 6: Examples of usual staining patterns for all HER2 IHC subgroups 0, 1+, 2+, and 3+, at (40X magnification)
Images courtesy of a contracted breast pathologist.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.
1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122.

HER2 evaluation framework

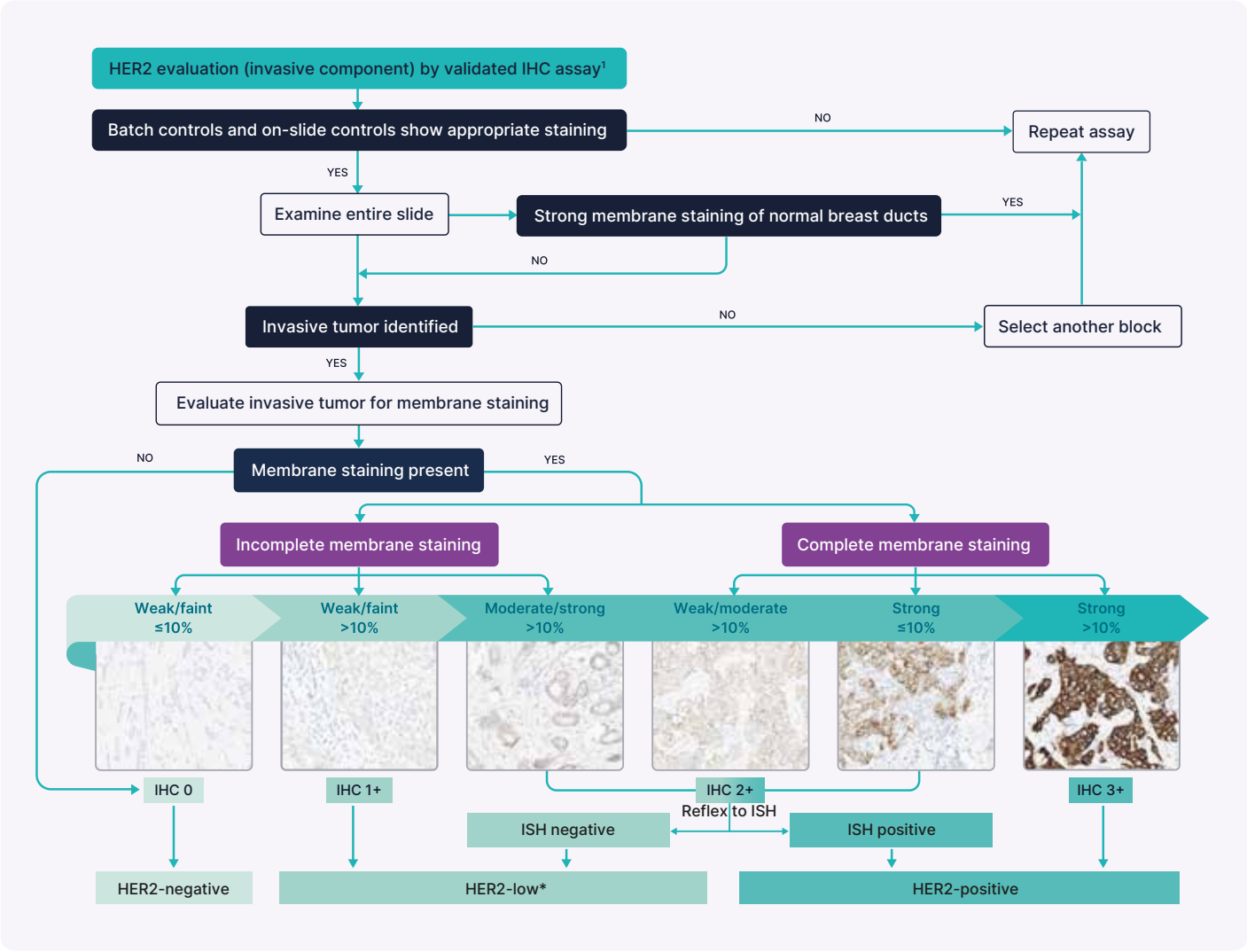


Figure 7: Proposed algorithm for interpreting HER2 (including HER2-low)

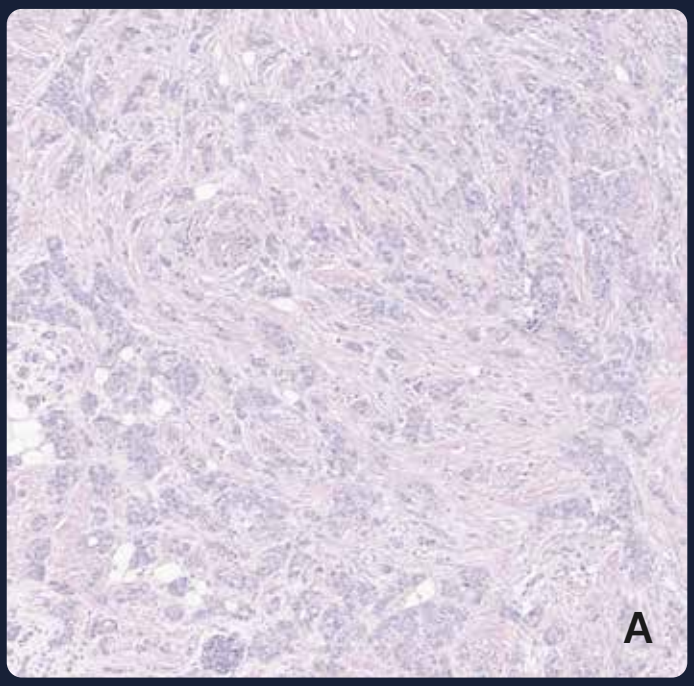
Evaluating pattern staining and intensity[1-5]

Incomplete membrane staining		**Complete membrane staining**	
Weak/ faint ≤10%	Faint/barely perceptible membrane staining observed in ≤10% of tumor cells. Staining hardly perceptible at **4X(5X)**** and **10X**, visible at **20X** and confirmed at **40X**	**Weak/ moderate >10%**	Weak to moderate complete membrane staining observed in >10% of tumor cells. Staining visible at **4X(5X)****, confirmed at **10-20X**
Weak/ faint >10%	Faint/barely perceptible incomplete membrane staining observed in >10% of tumor cells. Staining hardly perceptible at **4X(5X)**** and **10X**, visible at **20X** and confirmed at **40X**	**Strong ≤10%**	Strong complete membrane staining observed in ≤10% of tumor cells. Staining visible at **4X(5X)****, confirmed at **10-20X**
Moderate/ strong >10%	Moderate to strong incomplete membrane staining observed in >10% of tumor cells. Staining visible at **4X(5X)****, confirmed at **10-20X**	**Strong >10%**	Strong/intense, complete membrane staining observed in >10% of tumor cells and uniform 'chicken-wire' pattern. Staining visible at **4X(5X)****

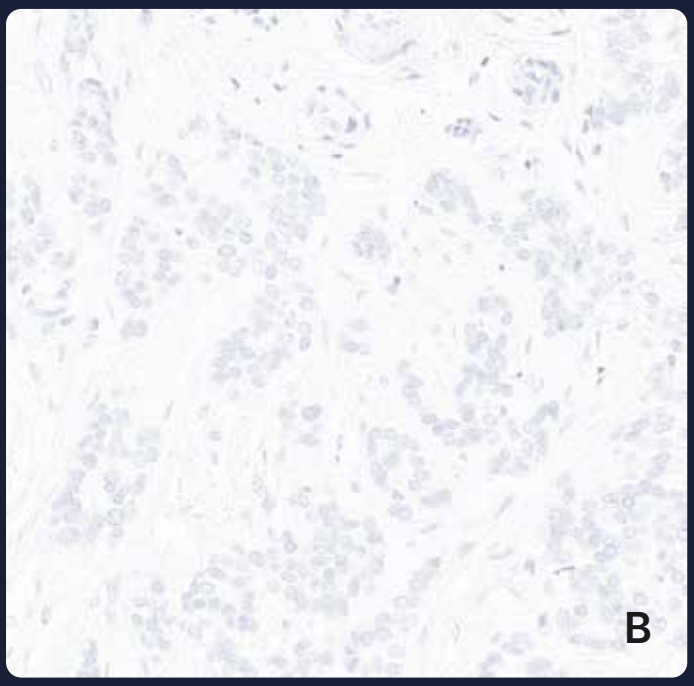
*HER2-low is not currently classified within the 2023 ASCO-CAP guideline update, but is recognized as HER2-negative.[6]
**Depending on the type of magnifiers available, pathologists will use 4X or 5X.
ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemsitry; ISH, *in situ* hybridization.
1. AstraZeneca and Daiichi Sankyo. Data on file. REF-18013. 2021. 2. Tarantino P et al. J Clin Oncol 2020;38(17):1951-1962. 3. Zhang H et al. Am J Clin Pathol 2021; ajab117:1-9. 4. AstraZeneca and Daiichi Sankyo. Data on file. REF-18100. 2022. 5. Franchet C et al. Annales de Pathologie 2021;41(6):507-520. 6. Wolff AC, et al. Arch Pathol Lab Med 2023. https://doi.org/10.5858/arpa.2023-0950-SA.

Staining patterns in clinical breast cases

IHC 0



Scan QR code to view the digital image on HER2Know.com



Scan QR code to view the digital image on HER2Know.com

CASE 1

(A) Well differentiated to moderately differentiated invasive ductal carcinoma of no special type (NST) with focal tubulo-lobular features with histologic grade (H&E stained section, 5X magnification).

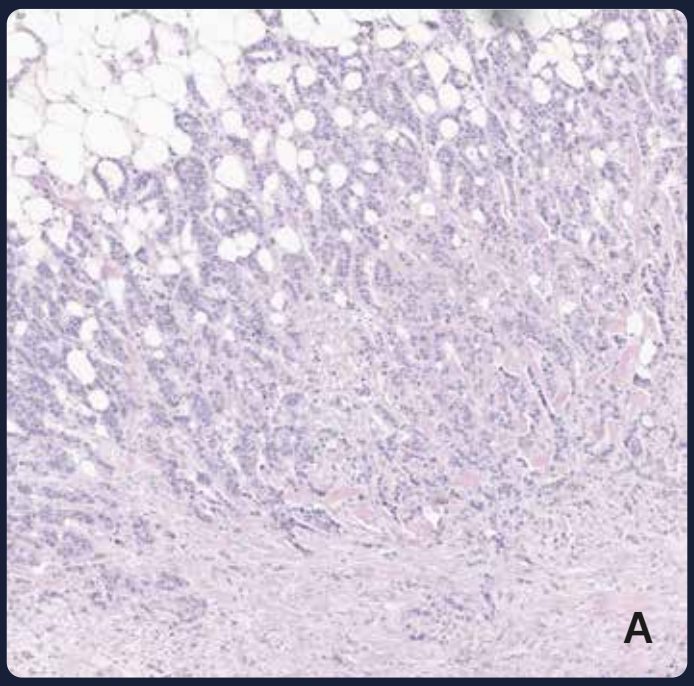
(B) HER2 IHC assay (20X original magnification). 100% of tumor cells with no staining.

IHC score: 0

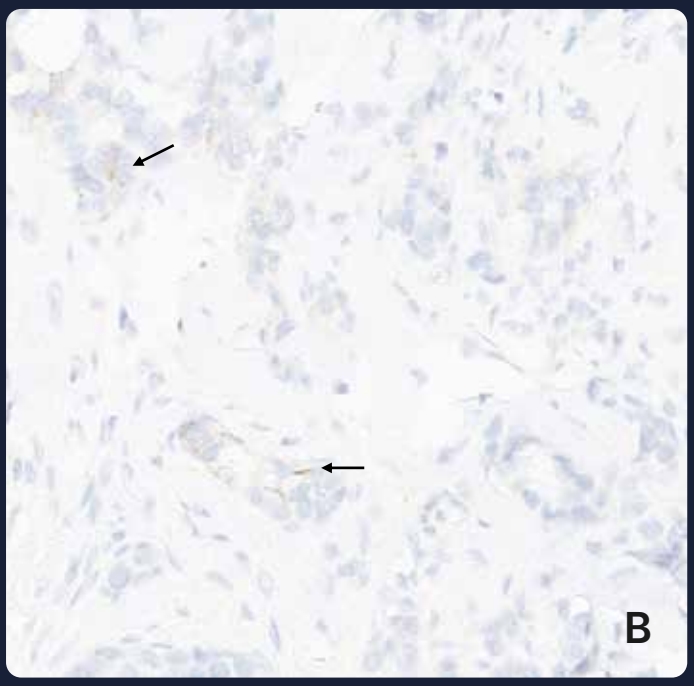
HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

IHC 0



Scan QR code to view the digital image on HER2Know.com



Scan QR code to view the digital image on HER2Know.com

CASE 2

(A) Moderately differentiated invasive ductal carcinoma of NST presenting a central area of highly desmoplastic stroma where the tumor takes some tubulo-lobular features, with histologic grade 2 (H&E stained section, 5X magnification).

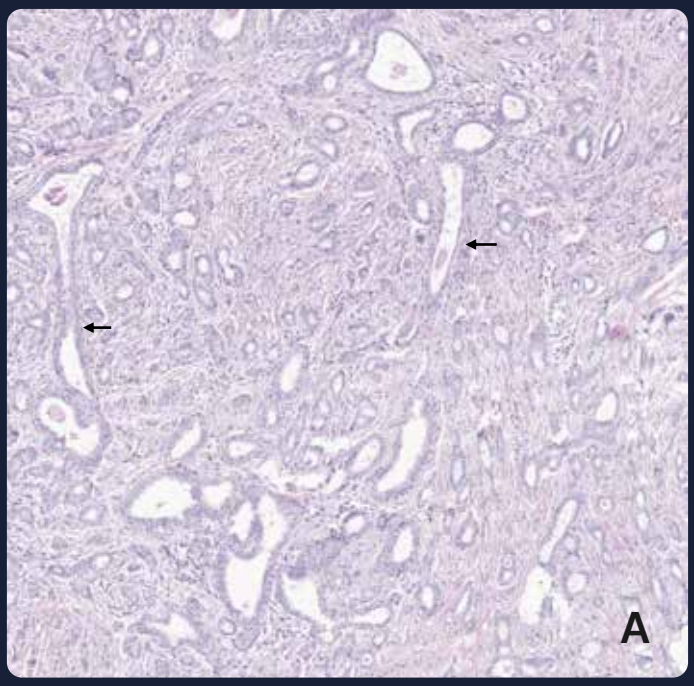
(B) HER2 IHC assay (20X original magnification). Less than 10% (about 3%) of tumor cells displaying a faint and incomplete membranous staining **(black arrows)**. A few cells of ductal breast carcinoma *in situ* (DCIS) are displaying HER2 staining and should not be included into the score.

IHC score: 0

DCIS, ductal carcinoma *in situ*; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

IHC 0



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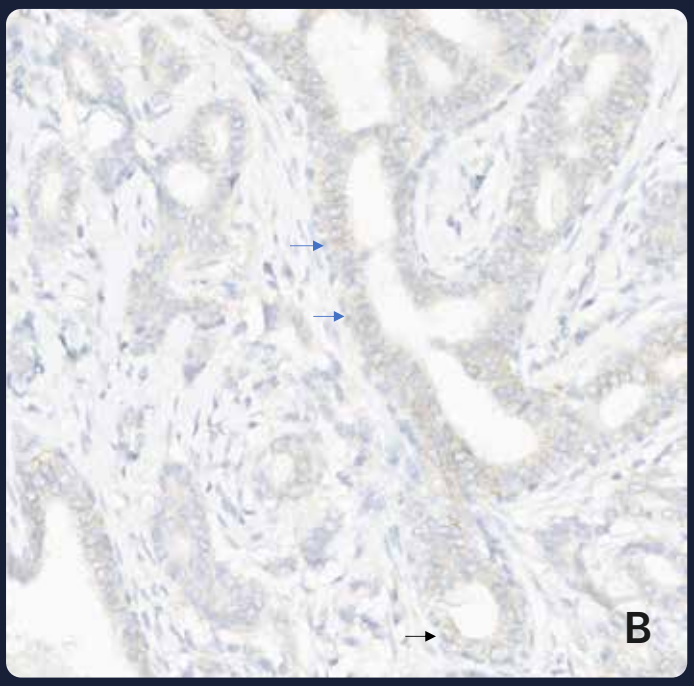

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CASE 3

(A) Well differentiated invasive tubular carcinoma with histologic grade 1. Notice the non-invasive ducts **(black arrows)** intercalated between the invasive tubules (H&E stained section, 5x magnification).

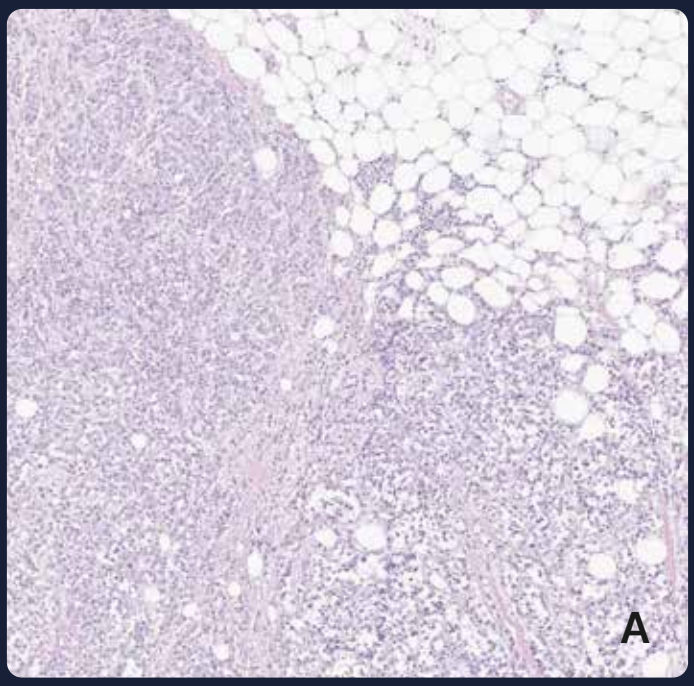
(B) HER2 IHC assay (20X original magnification). The tumor displays an incomplete and faint membranous staining **(black arrow)** in about 10% of the tumor cells falling short from an IHC 1+ score. Many non-invasive ducts are intercalated between the tumor tubules and display a membranous staining **(blue arrows)** not to be confused with the invasive component that is mainly negative.

IHC score: 0

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

IHC 1+



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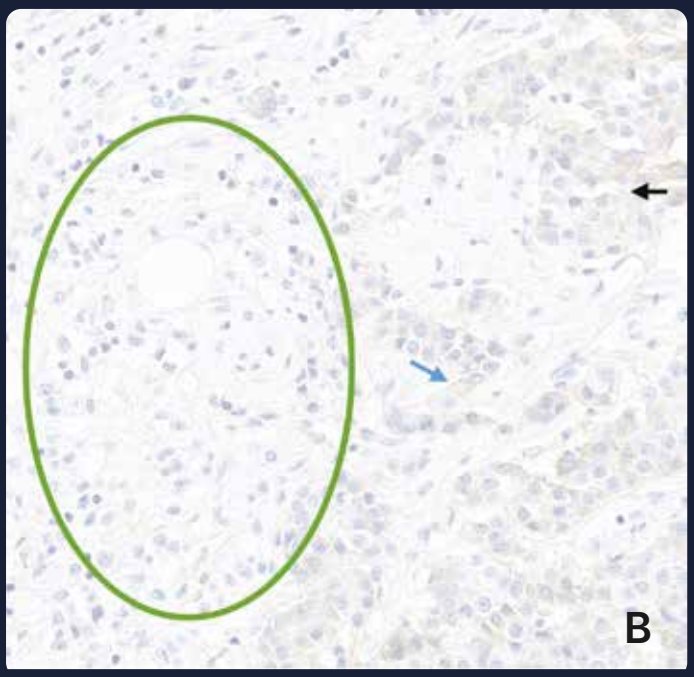

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CASE 4

(A) Moderately differentiated invasive ductal carcinoma of NST with histologic grade 2 (H&E stained section, 5X magnification).

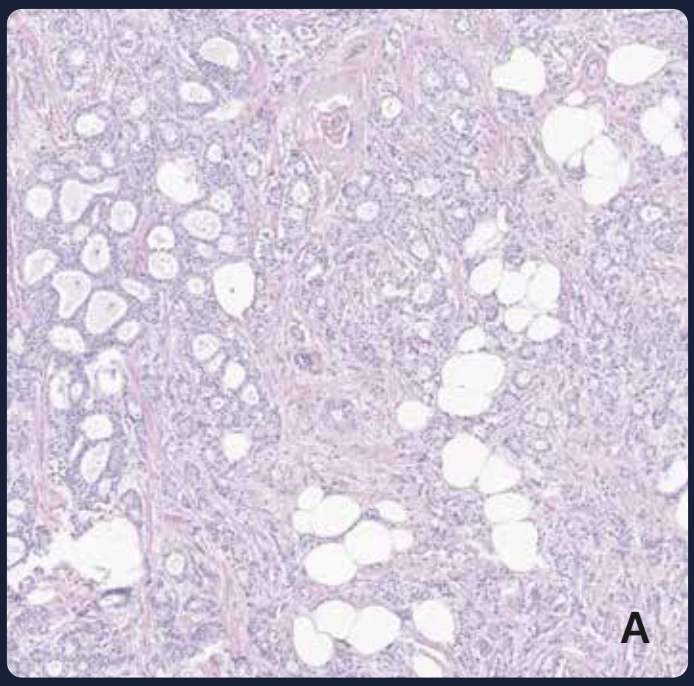
(B) HER2 IHC assay (20X original magnification). About 15% of the tumor cells form a distinct focus displaying a faint and incomplete membranous staining **(black arrow)** while the rest of the tumor is negative **(green circle)**. Some of the tumor cells display a non-specific, faint cytoplasmic blush **(blue arrow)** which does not affect evaluation.

IHC score: 1+

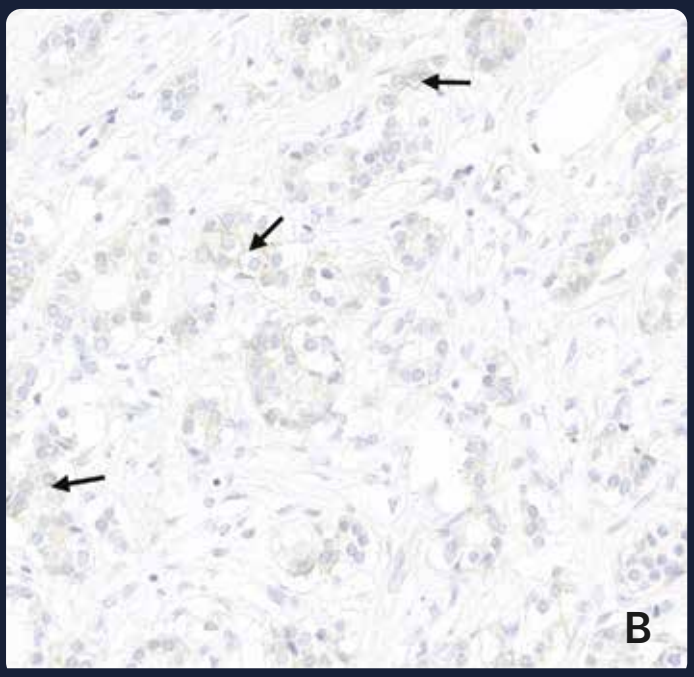
HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

IHC 1+



Scan QR code to view the digital image on HER2Know.com



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CASE 5

(A) Well differentiated invasive cribriform carcinoma (ICC) with histologic grade 1 (H&E stained section, 5X magnification).

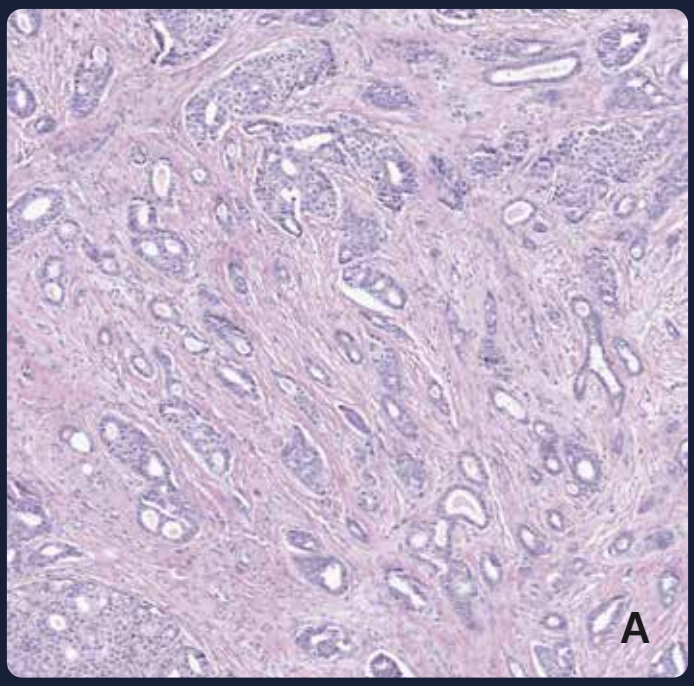
(B) HER2 IHC assay (20X original magnification). 40-50% of the tumor cells are diffusely spread throughout the tumor and display a faint and incomplete membranous staining **(black arrows).**

IHC score: 1+

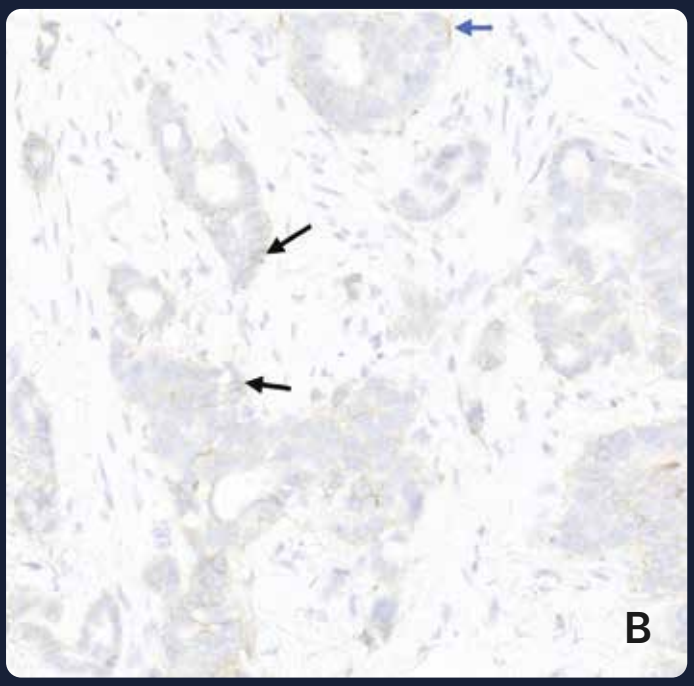
HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; ICC, invasive cribriform carcinoma; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

IHC 1+



Scan QR code to view the digital image on HER2Know.com



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CASE 6

(A) Well differentiated ICC with histologic grade 1 (H&E stained section, 5X magnification).

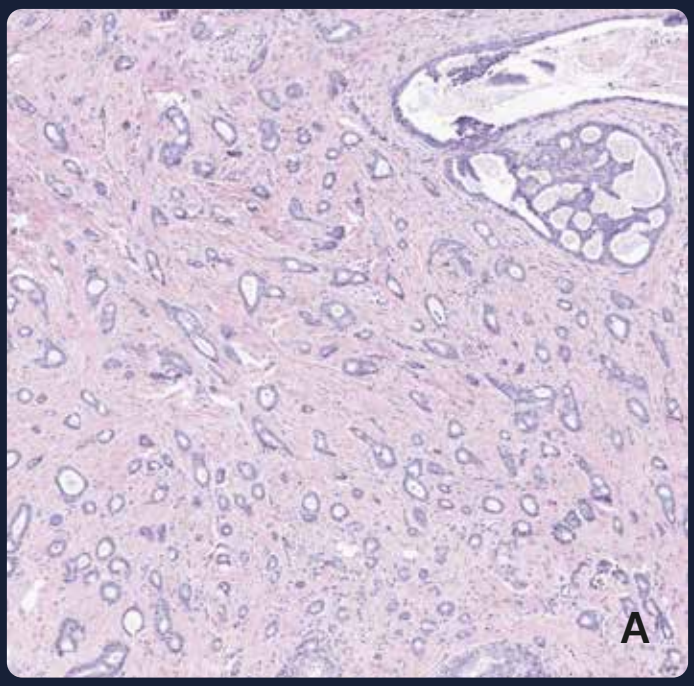
(B) HER2 IHC assay (20X original magnification). Barely more than 10% of tumor cells display a faint and incomplete membranous staining **(black arrows)**. In some areas, non-specific basal staining is identified in a few tumor cells **(blue arrow)** and should not be considered into the final score. This is a borderline case between IHC 0 and 1+.

IHC score: 1+

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; ICC, invasive cribriform carcinoma; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

IHC 1+



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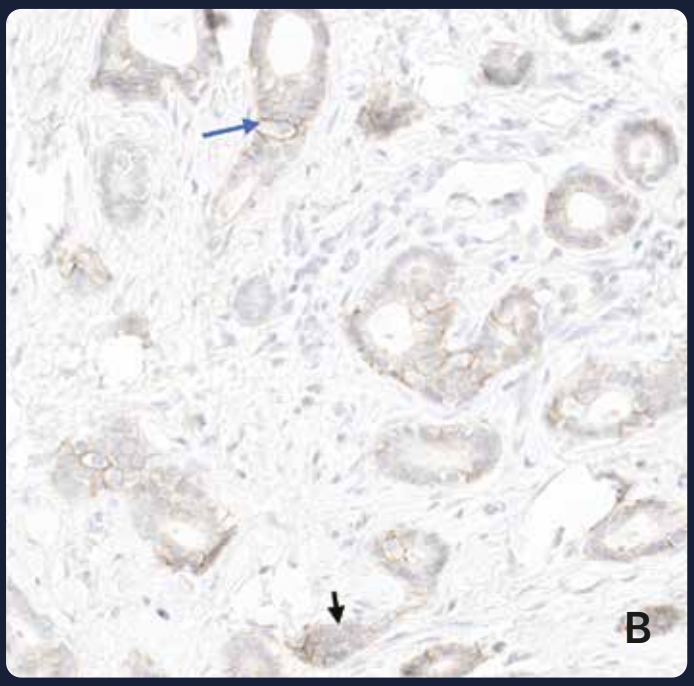

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CASE 7

(A) Well differentiated invasive tubular carcinoma with atypical duct hyperplasia with histologic grade 1 (H&E stained section, 5X magnification).

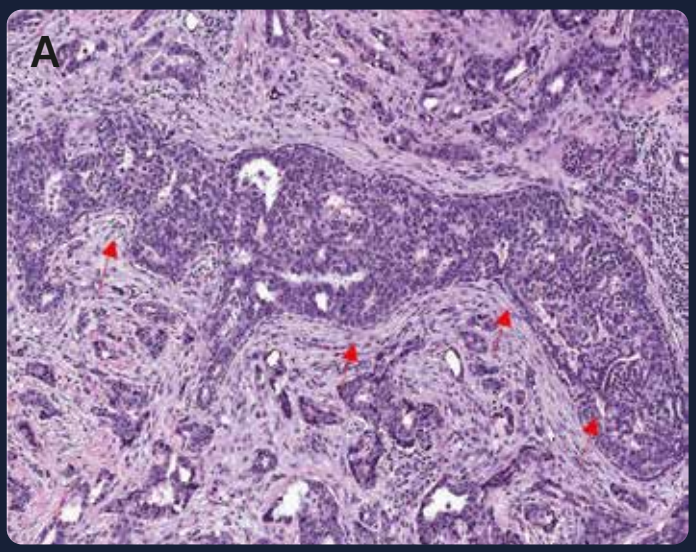
(B) HER2 IHC assay (20X original magnification). About 70% of tumor cells display an incomplete and faint membranous staining **(black arrow)**. A few of them display a rather moderate to intense basolateral and lateral staining **(blue arrow)** rendering the diagnosis quite challenging between an IHC 1+ and 2+ score. Careful analysis does not identify the IHC 2+ staining pattern in more than 10% of the tumor cells. This borderline case is kept at an IHC 1+ score.

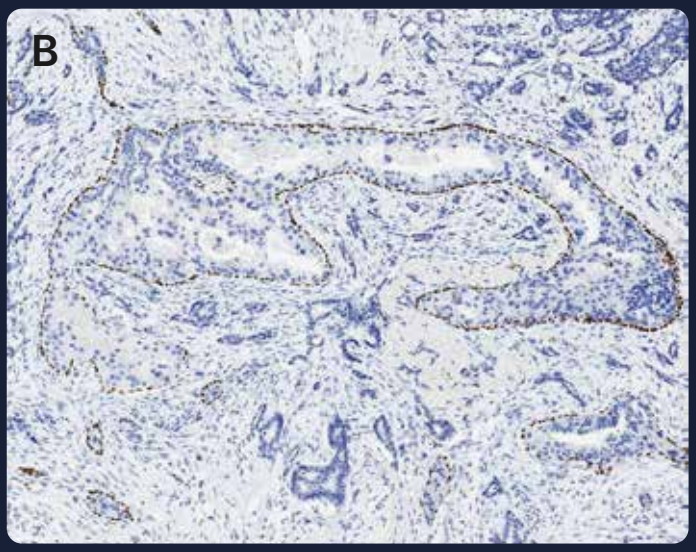
IHC score: 1+

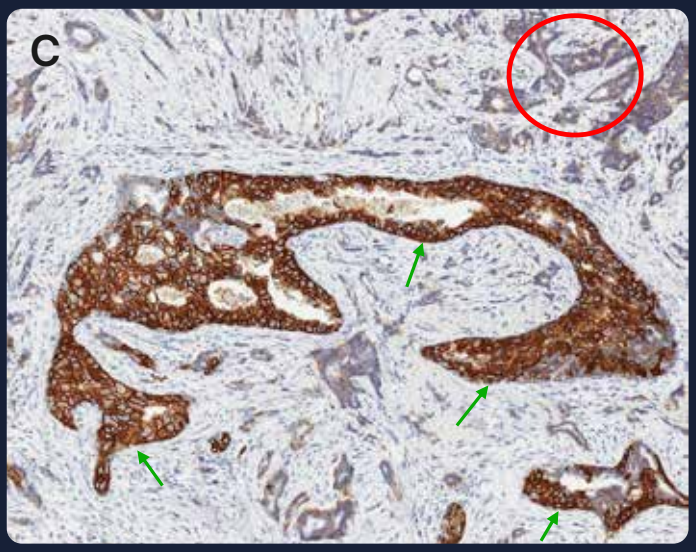
HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

IHC 1+







CASE 8

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(A) Moderately differentiated invasive ductal carcinoma with histologic grade 2 (H&E stained section, 20X magnification). **Red arrows** indicate DCIS.

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(B) P63 stain decorates the myoepithelial cell layer in DCIS (corresponds to the duct with **red arrows** in Figure A).

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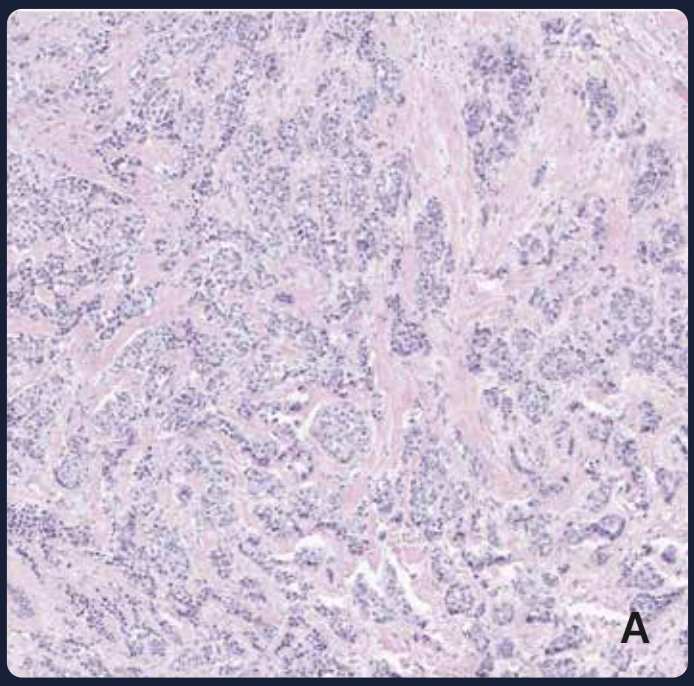
(C) HER2 IHC assay (20X original magnification). Weak staining intensity with 50% of tumor cells **(red circle)**. DCIS positive for HER2 **(green arrows)**. Note, this could be a pitfall in labs that perform FISH with no IHC. When this pattern of staining is recognized, retrospective staining with myoepithelial markers could be useful.

DCIS, ductal carcinoma *in situ*; FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; P63, tumor protein 63.

Staining patterns in clinical breast cases

IHC 2+



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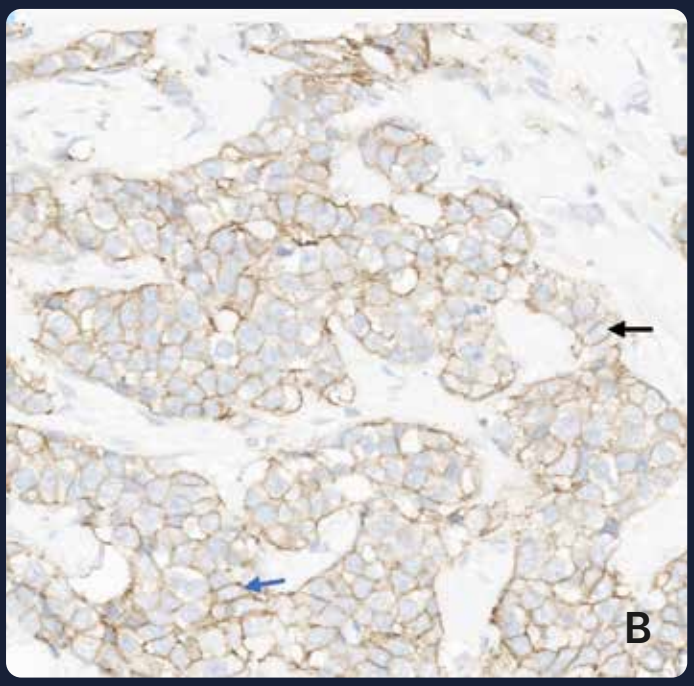

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CASE 9

(A) Poorly differentiated invasive ductal carcinoma of NST with histologic grade 3 (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). About 95% of tumor cells display a weak **(black arrow)** to moderate **(blue arrow)** complete membranous staining homogeneously distributed throughout the entire tumor with only a few of them strongly stained.

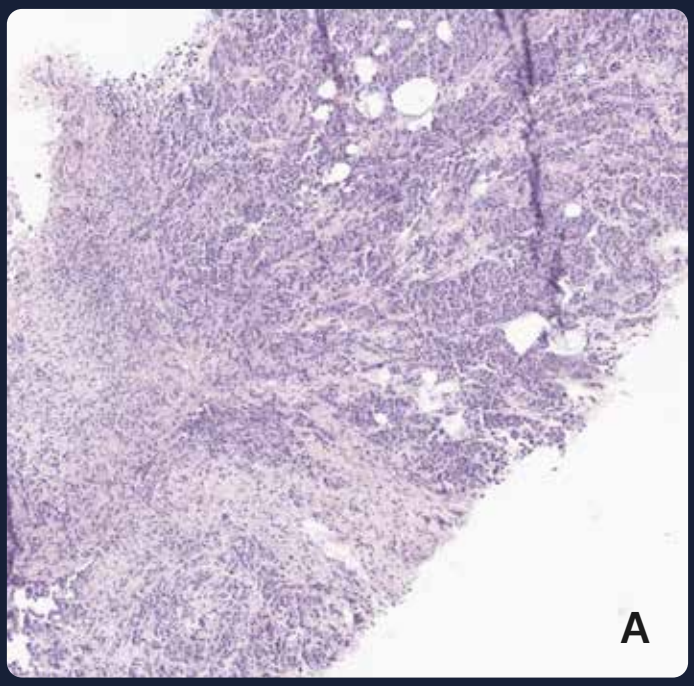
IHC score: 2+

This is an equivocal case that needs additional testing using an ISH/FISH method to identify potential HER2 amplification.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Staining patterns in clinical breast cases

IHC 2+



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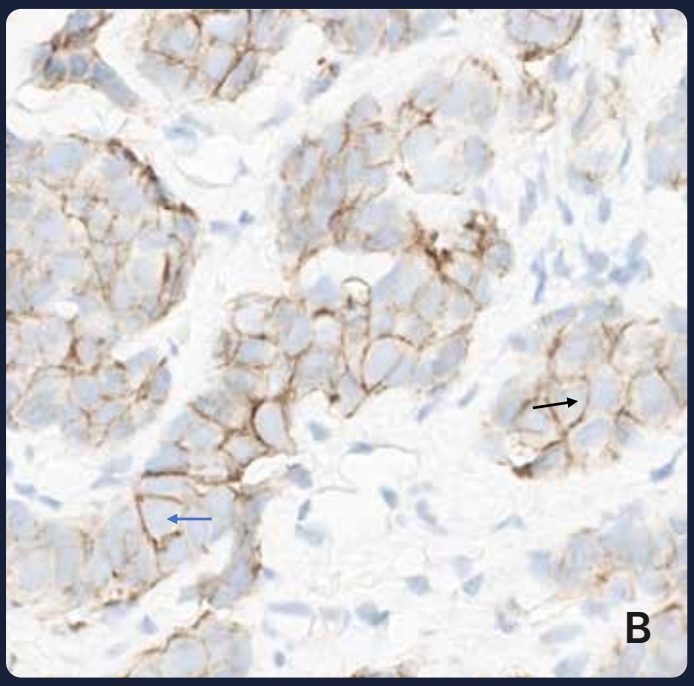

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CASE 10

(A) Poorly differentiated invasive ductal carcinoma of NST with histologic grade 3 (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). About 95% of tumor cells display a weak **(black arrow)** to moderate **(blue arrow)** complete membranous staining homogeneously distributed throughout the entire tumor, with only a few of them strongly stained.

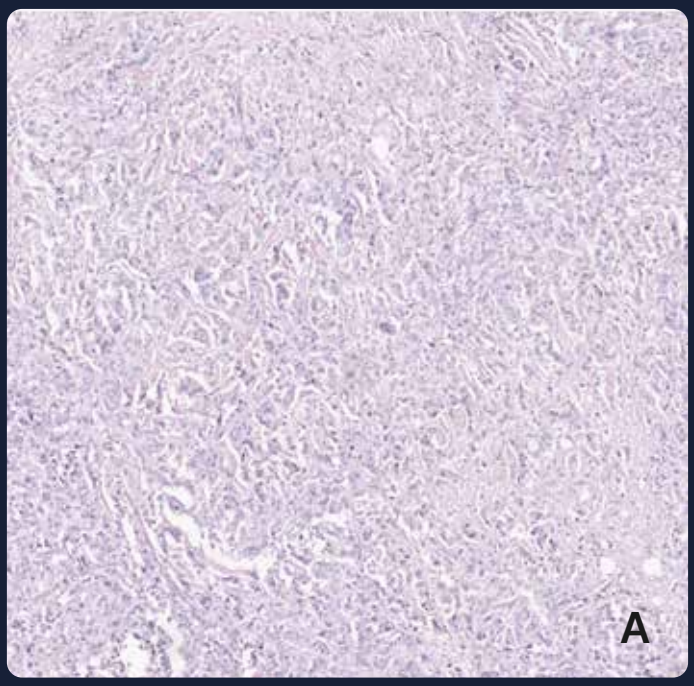
IHC score: 2+

This case has an equivocal status that needs further evaluation using an ISH/FISH method to identify potential HER2 amplification.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Staining patterns in clinical breast cases

IHC 2+



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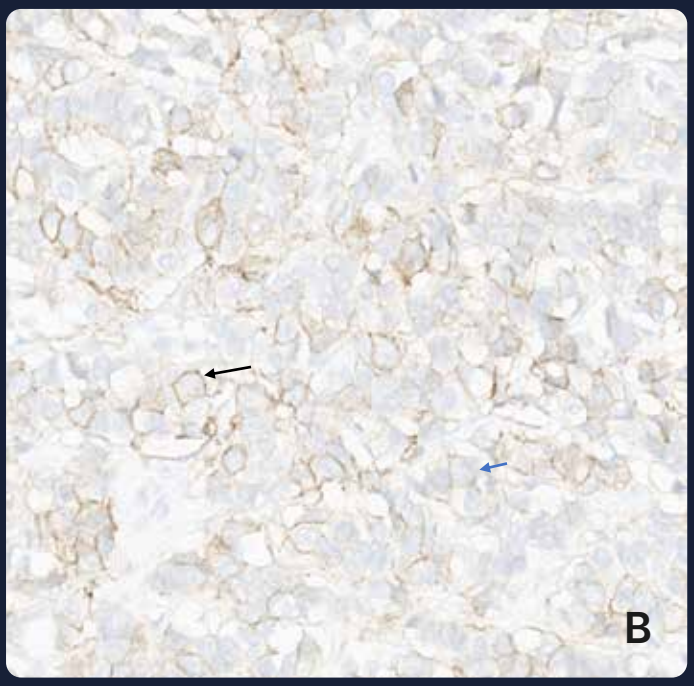

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CASE 11

(A) Poorly differentiated invasive ductal carcinoma of NST with histologic grade 3 (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). About 70% of tumor cells display a weak **(blue arrow)** to moderate **(black arrow)** complete membranous staining.

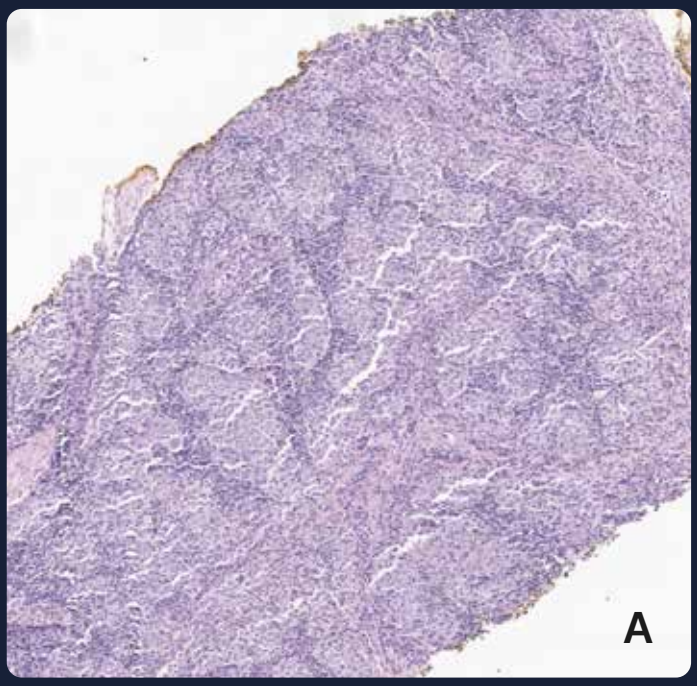
IHC score: 2+

This is an equivocal case where an additional testing using an ISH/FISH method is recommended to identify HER2 amplification, especially when considering the high grade of this tumor.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Staining patterns in clinical breast cases

IHC 2+



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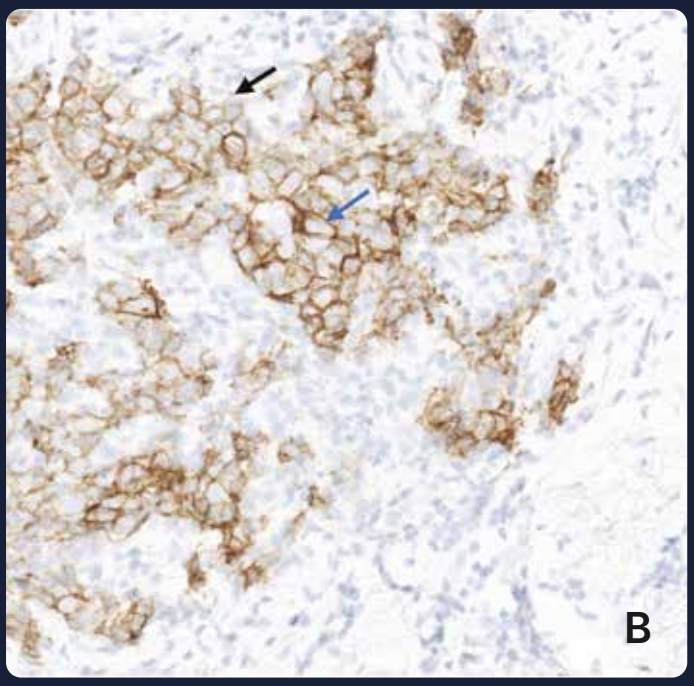

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CASE 12

(A) Moderately differentiated invasive ductal carcinoma of NST with histologic grade 2, rich in tumor-infiltrating lymphocytes (TIL) (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). Tumor cells display a complete membranous staining of moderate intensity **(black arrow)** throughout the tumor, with the exception of a few areas where the staining intensity is stronger **(blue arrow)**. Due to the intermingled staining intensities even within the same tumor nest, it is difficult to ascertain the presence of the strong staining in more than 10% of the tumor cells.

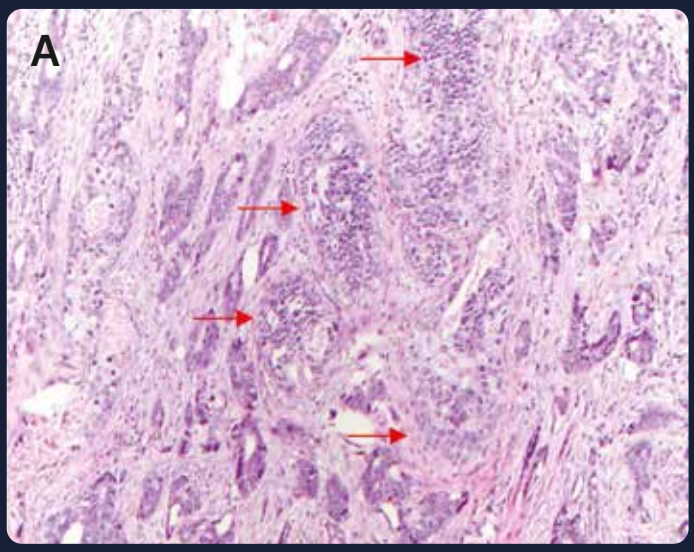
IHC score: 2+

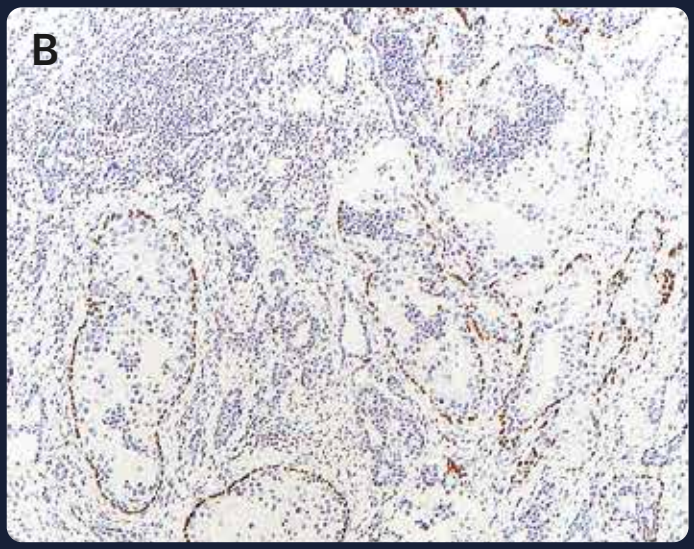
This borderline case is therefore cautiously kept at a score of IHC 2+ with the recommendation of running additional testing using an ISH/FISH method to identify HER2 amplification.

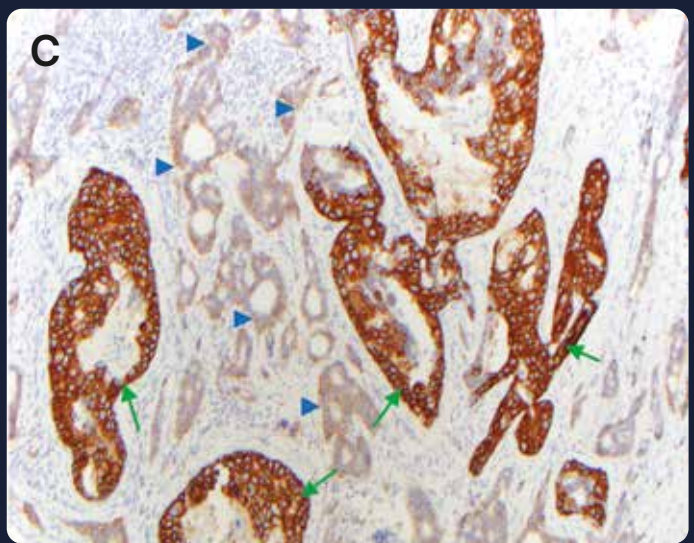
FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type; TIL, tumor-infiltrating lymphocytes.

Staining patterns in clinical breast cases

IHC 2+







CASE 13

This is an equivocal case where an additional testing using an ISH/FISH method is recommended to identify HER2 amplification especially when considering the high grade of this tumor.

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(A) Moderately differentiated invasive ductal carcinoma with histologic grade 2 (H&E stained section, 20X magnification). **Red arrows** indicate DCIS.

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(B) P63 stain decorates the myoepithelial cell layer in DCIS (corresponds to the duct with **red arrows** in Figure A).

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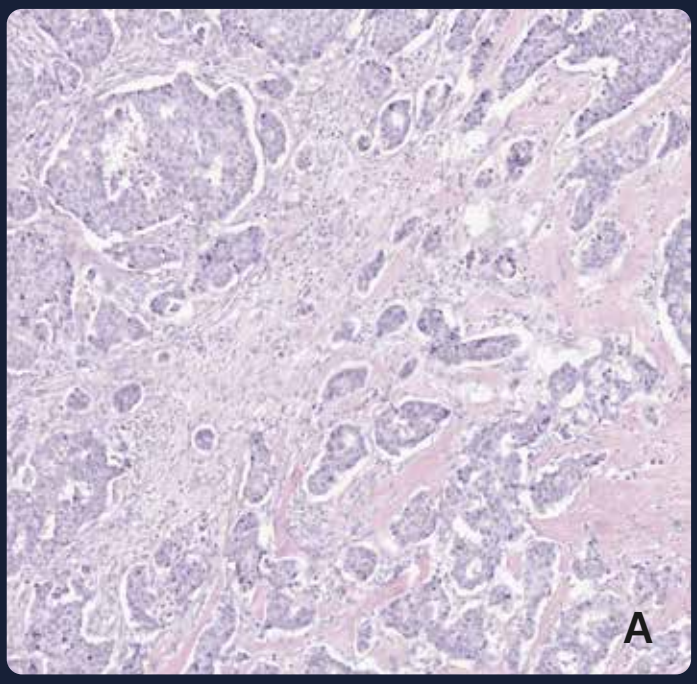
(C) HER2 IHC assay (20X original magnification). Moderate staining intensity with 50% of tumor cells **(blue arrowheads)**. DCIS positive for HER2 **(green arrows)**. Note, this could be a pitfall in labs that perform FISH with no IHC. When this pattern of staining is recognized, retrospective staining with myoepithelial markers could be useful.

DCIS, ductal carcinoma *in situ*; FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; P63, tumor protein 63.

Staining patterns in clinical breast cases

IHC 3+



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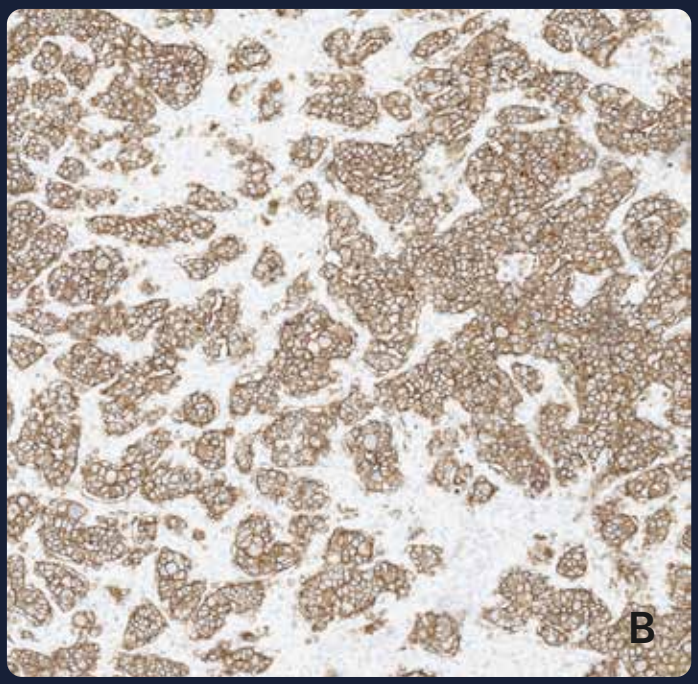

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CASE 14

(A) Moderately differentiated to poorly differentiated invasive ductal carcinoma of NST with focal necrosis and peritumoral stromal retraction with histologic grade 3 (H&E stained section, 5X original magnification).

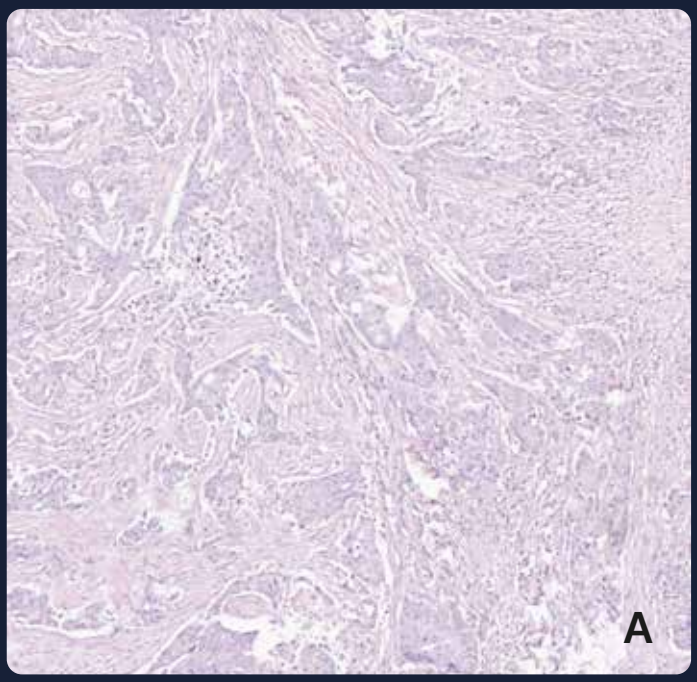
(B) HER2 IHC assay (5X original magnification). About 100% of tumor cells display a complete and strong membranous staining consistent with the 'chicken wire' pattern identifiable at low magnification. This pattern is highly specific for HER2 overexpression.

IHC score: 3+

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

IHC 3+



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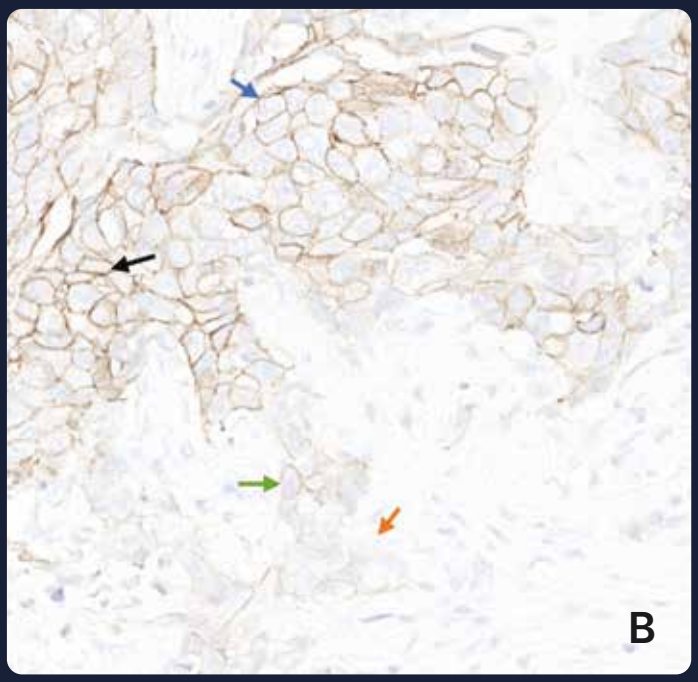

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CASE 15

(A) Poorly differentiated invasive ductal carcinoma of NST with focal necrosis and peritumoral stromal retraction with histologic grade 3 (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). Tumor cells display a heterogenous membranous staining spanning a range of patterns and intensities from strong complete **(25% black arrow)** to moderate complete **(50% blue arrow)** and weak incomplete **(20% green arrow)**. Some tumor cells are completely negative **(5% orange arrow)**. Because strong and complete 'chicken wire' pattern is identified in more than 10% of tumor cells, the overall score is IHC 3+.

IHC score: 3+

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

Summary

Membrane staining intensity and pattern should be evaluated when determining HER2 expression and only the invasive (infiltrating) breast carcinoma should be scored.[1] This also applies to cases displaying DCIS admixed with invasive ductal carcinoma component.[2] In such cases, the corresponding H&E should always be reviewed.[2] Myoepithelial cell markers can be diagnostically useful in the distinction of many benign, *in situ*, and invasive lesions.[3]

Clinical cases with considerable staining of normal breast components should be excluded from IHC interpretation.[1]

Multiple variables impact HER2 test interpretation, hence evaluation of HER2 requires recognition of diagnostic pitfalls and staining patterns susceptible to misinterpretation.[4]

The final score should be determined based on the criteria for staining pattern and intensity, as per the ASCO-CAP guidelines. 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.[1]

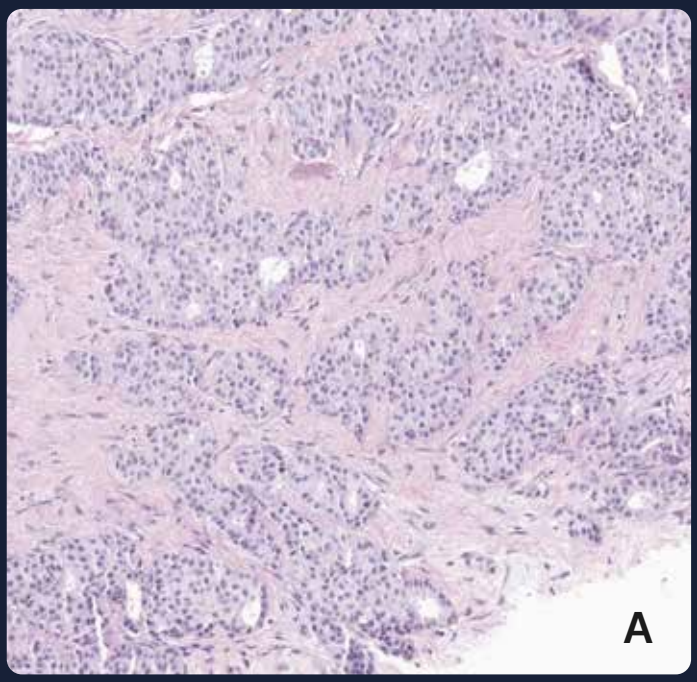
The next few cases will discuss staining artifacts that may prove challenging to interpret in clinical practice, and how to evaluate their staining pattern and intensity.

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; DCIS, ductal carcinoma *in situ*; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization.

1. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 2. Wolff AC, et al. J Clin Oncol 2007; 25(1):118-145. 3. Corben AD, et al Surg Pathol Clin 2009;2(2):351-373. 4. Kim SW, et al. J Pathol Transl Med 2016;50:411-418.

Staining patterns in clinical breast cases

Artifacts



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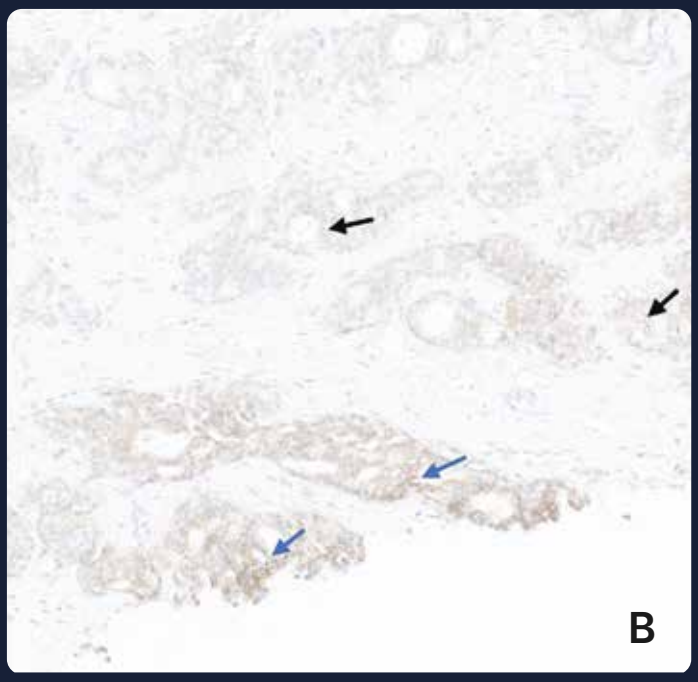

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CASE 16

(A) Well differentiated ICC with histologic grade 1 (H&E stained section, 5X magnification).

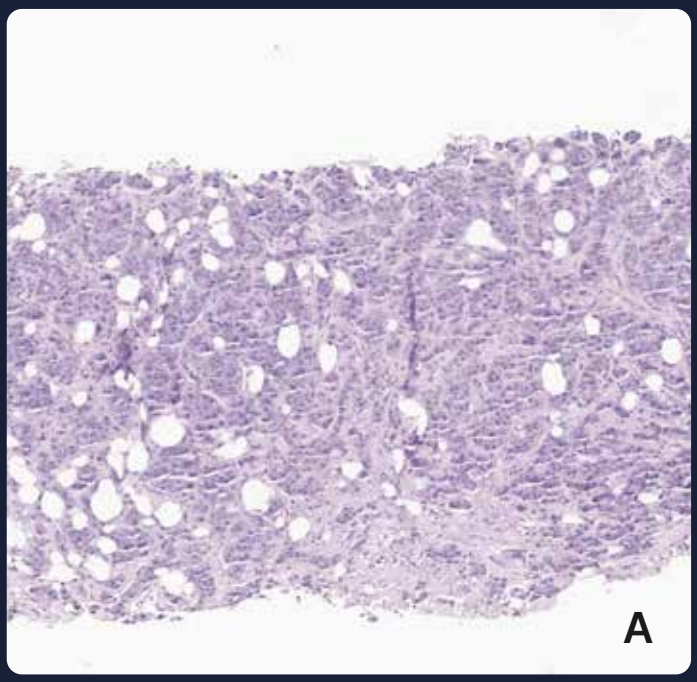
(B) HER2 IHC assay (5X original magnification). Most tumor cells display weak to moderate incomplete membranous staining **(black arrows)**, except for the ones located at the edge of the tissue core that have a stronger staining intensity **(blue arrows)** with even a complete membranous pattern in some of them. This abrupt difference in staining is a well-known artifact called edge effect, and should not mislead the pathologist who might be tempted to upgrade the score on this well-differentiated carcinoma. If in doubt, the use of a different testing method ISH/FISH could still be possible.

IHC score: 1+

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; ICC, invasive cribriform carcinoma; IHC, immunohistochemistry; ISH, *in situ* hybridization.

Staining patterns in clinical breast cases

Artifacts



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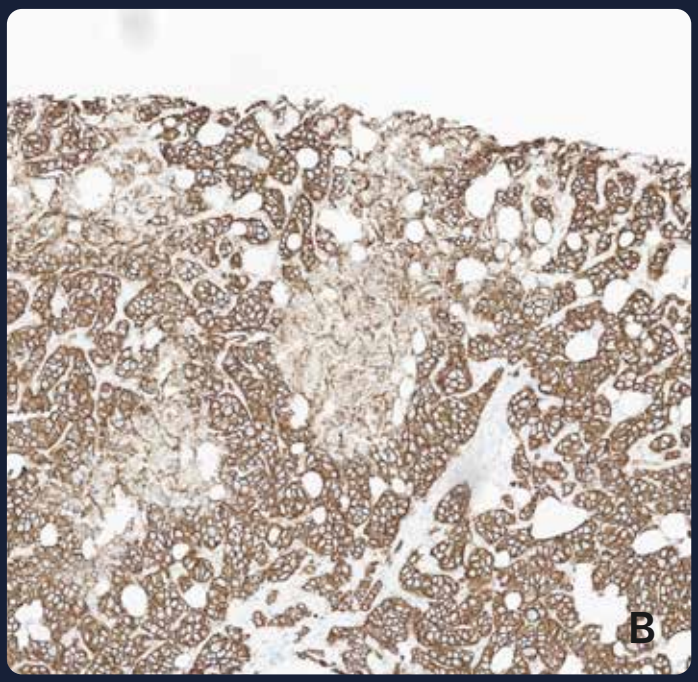

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CASE 17

(A) Moderately differentiated invasive ductal carcinoma of NST with histologic grade 2 (H&E stained section, 5X original magnification).

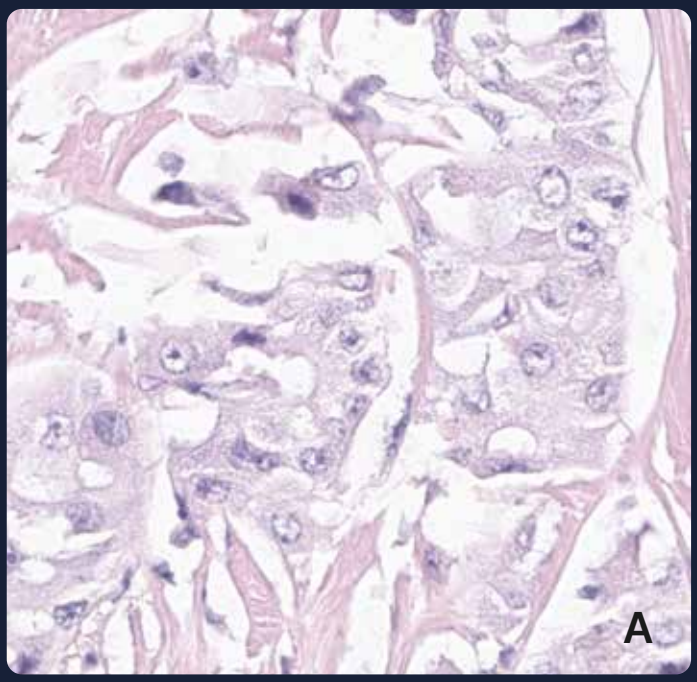
(B) HER2 IHC assay (5X original magnification). A fixation artifact caused a patchy staining of tumor cells mimicking a heterogeneity. While most tumor cells display a strong and complete membranous staining, we see patches of tumor cells where the staining is abruptly decreased with blurred cellular contours. This is a false heterogeneous pattern. Since more than 50% of the tumor is adequately stained allowing to reach an appropriate score, no repeat is needed in this case.

IHC score: 3+

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

Artifacts



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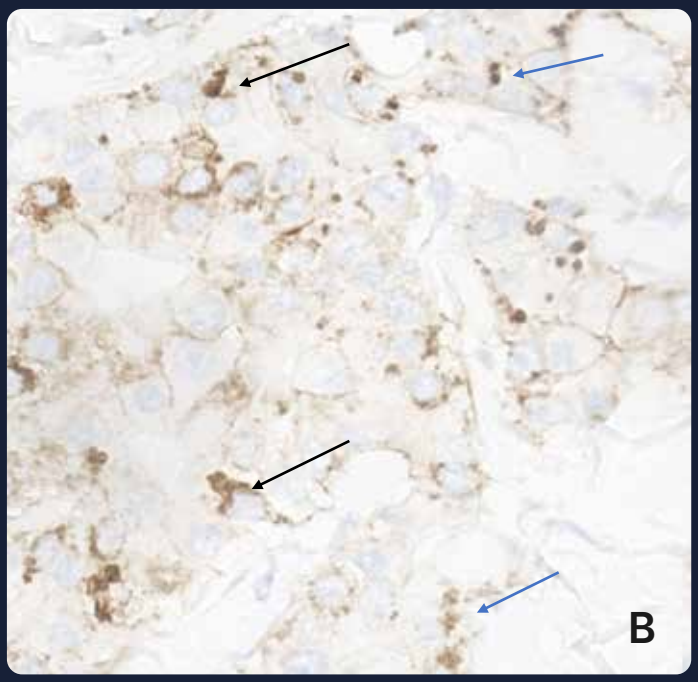

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CASE 18

(A) Moderately differentiated duct carcinoma with NST where a probable delay to fixation has caused staining artifacts with cytoplasmic coarse granules and blurred membranous contours (H&E stained section, 40X original magnification).

(B) HER2 IHC assay (40X original magnification). Suboptimal staining is noticed in multiple tumor cells with a nonspecific coarse granular cytoplasmic staining **(black arrows)** obscuring the already fading cellular membranes **(blue arrows)**. In better preserved tumor cells and *in situ* components (not seen on this picture), a specific staining is identified where tumor cells display a mainly incomplete weak to moderate membranous staining. This case would benefit from either an IHC repeat on a better fixed tissue section and/or a different testing method (ISH/FISH) if the staining artifact would persist.

IHC score: cannot be determined (indeterminate)

Note: this case has been scored IHC 1+ and 2+ by different pathologists.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

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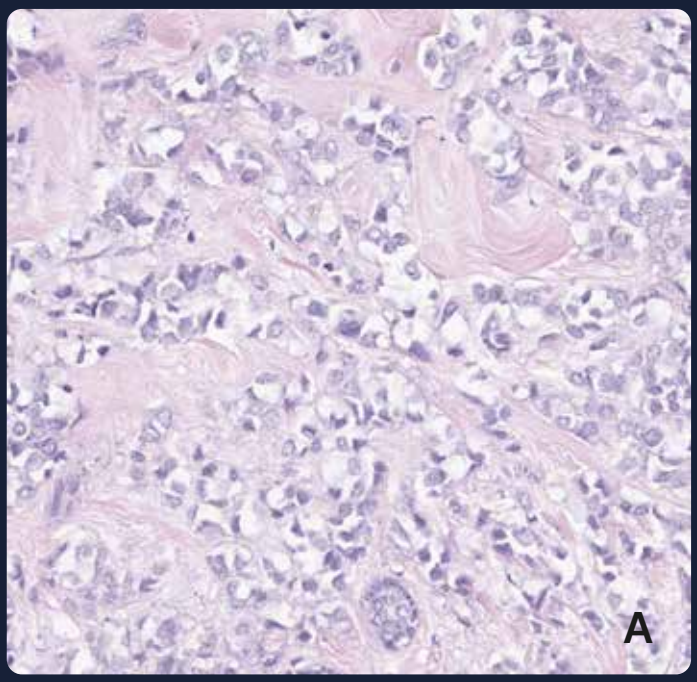
Artifacts



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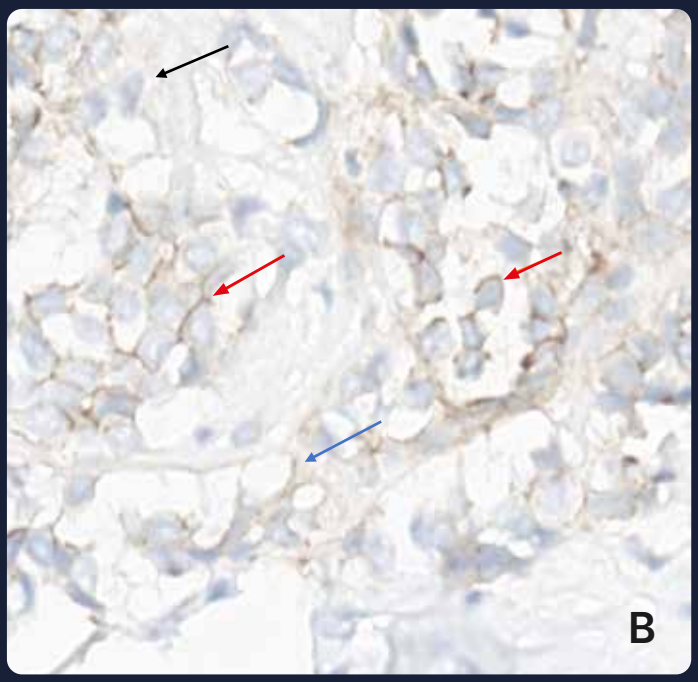

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CASE 19

(A) Moderately differentiated ductal carcinoma of NST and features of poor fixation affecting the nuclear size of tumor cells and increasing their pleomorphism (H&E stained section, 20X original magnification).

(B) HER2 IHC assay (40X original magnification). Poorly fixed tumor cells express an array of membranous staining from totally negative **(black arrow)** to faint/barely perceptible and incomplete **(blue arrow)**. However, more than 10% of tumor cells display an unusual moderate to intense basolateral/lateral **(red arrows)** staining pattern. This case qualifies for an equivocal status, that needs further evaluation using an ISH/FISH method to rule out a potential HER2 amplification.

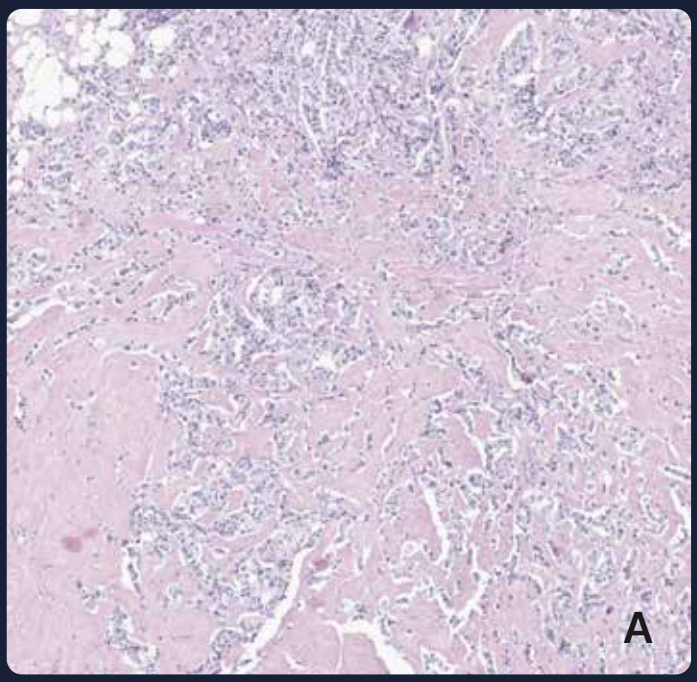
IHC score: 2+

Note: some pathologists scored this cases as IHC 1+. The impact of the poor fixation on this potentially amplified tumor (pleomorphic lobular) should be considered carefully.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Staining patterns in clinical breast cases

Artifacts



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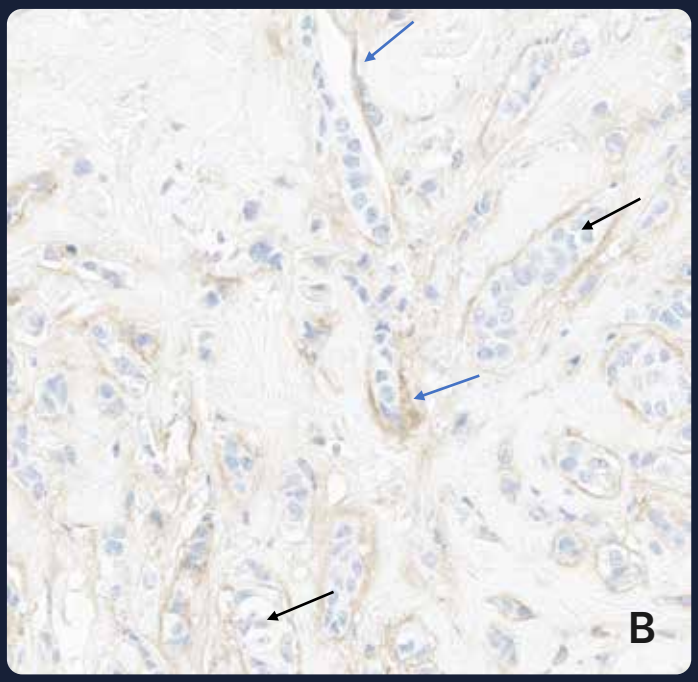

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CASE 20

(A) Moderately differentiated duct carcinoma with NST with highly sclerotic stroma creating retraction artifacts also known as peritumoral clefts where tumor elements are separated from the surrounding stroma (H&E stained section, 5X original magnification).

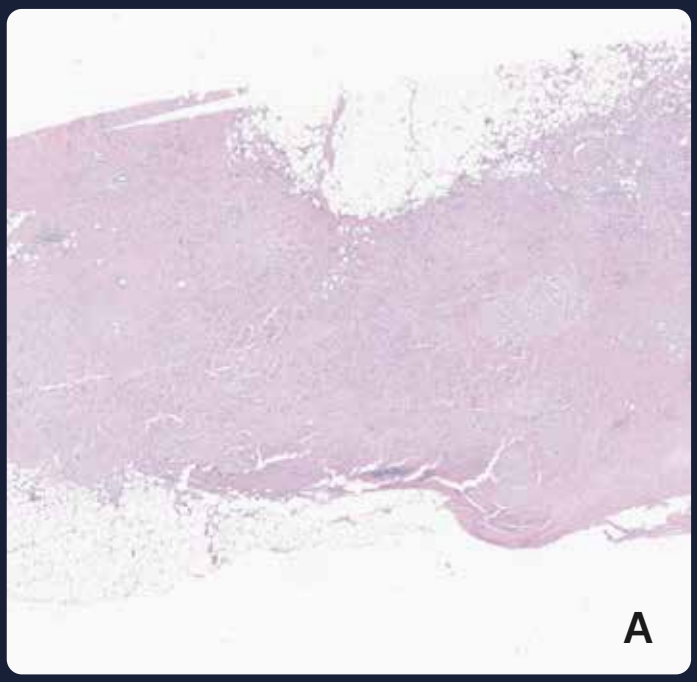
(B) HER2 IHC assay (20X original magnification). While tumors cells are extensively negative for specific membranous staining **(black arrows)**, we can identify a 3,3′-Diaminobenzidine (DAB) entrapment in the stroma at the edge of the stromal clefts **(blue arrows)**, creating a false impression of tumor cell basal staining. This is a non-specific background staining that should not be confused with a specific membranous staining. Elsewhere on the slide and at the edge of the tissue specimen, we can also see a few tumor cells (<10%) displaying both specific membranous and non-specific cytoplasmic staining more probably due to an added edge effect.

IHC score: 0

DAB, 3′3-Diaminobenzidine; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

Artifacts



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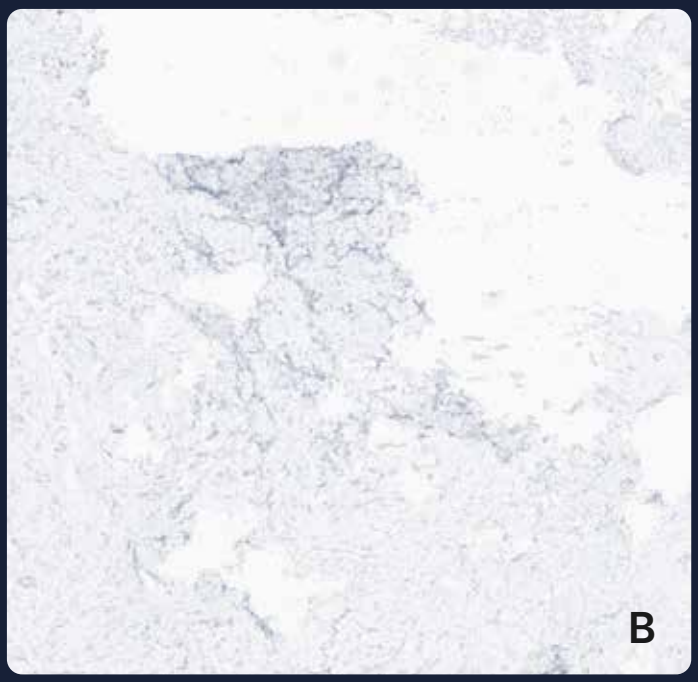

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CASE 21

(A) Well differentiated to moderately differentiated lobular carcinoma of classic type involving most of the tissue specimen (H&E stained section, original magnification).

(B) HER2 IHC assay (2.5X original magnification). Highly altered tissue section with both folding and partial tissue loss artifacts involving about 50% of the tissue specimen. Knowing that slightly more than 10% of tumor cells could shift the diagnostic from one score to a different one, we believe that it would be more cautious to repeat the IHC stain on a different and better-preserved tissue section allowing a more accurate assessment.

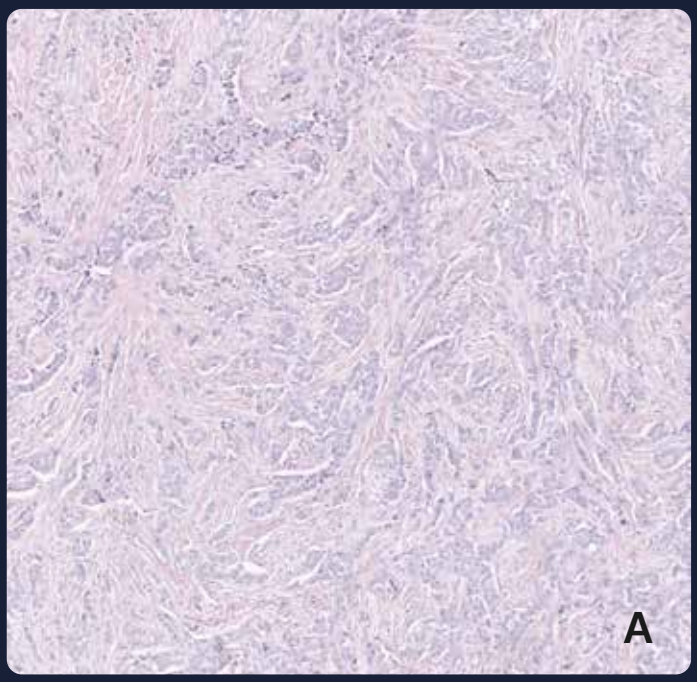
IHC score: indeterminate (cannot be determined)

Note: some pathologists gave a score of IHC 0 to this case.

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

Staining patterns in clinical breast cases

Artifacts



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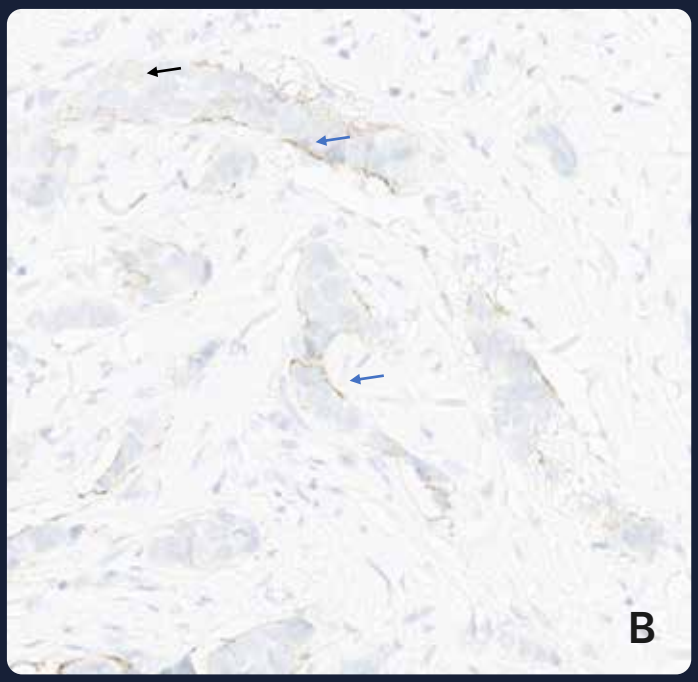

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CASE 22

(A) Well differentiated to moderately differentiated invasive ductal carcinoma of NST with tubulo-lobular features and stromal retraction around tumor nests with histologic grade 2 (H&E stained section, 5X magnification).

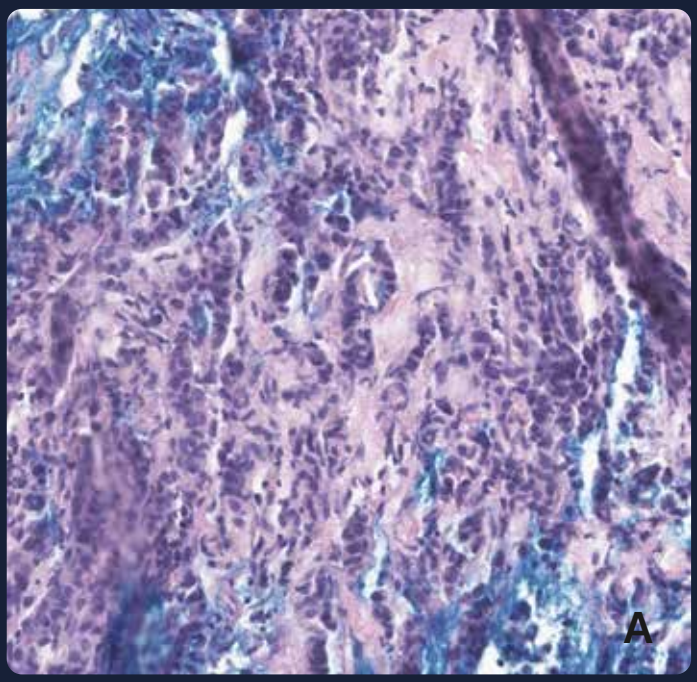
(B) HER2 IHC assay (20X original magnification). About 75% of tumor cells display an incomplete and faint membranous staining **(black arrow)** while a few of them display a stronger 'basal only' staining **(blue arrows)**. Careful review identifies the 'basal only' staining in areas where tumor nests are separated form their retracted surrounding stroma. This so-called retraction artifact is known to cause a 'basal only' staining artifact that should not be confused with a basolateral and lateral staining. The 'basal only' staining is not included in the score.

IHC score: 1+

HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; NST, no special type.

Staining patterns in clinical breast cases

Indeterminate (cannot be determined)



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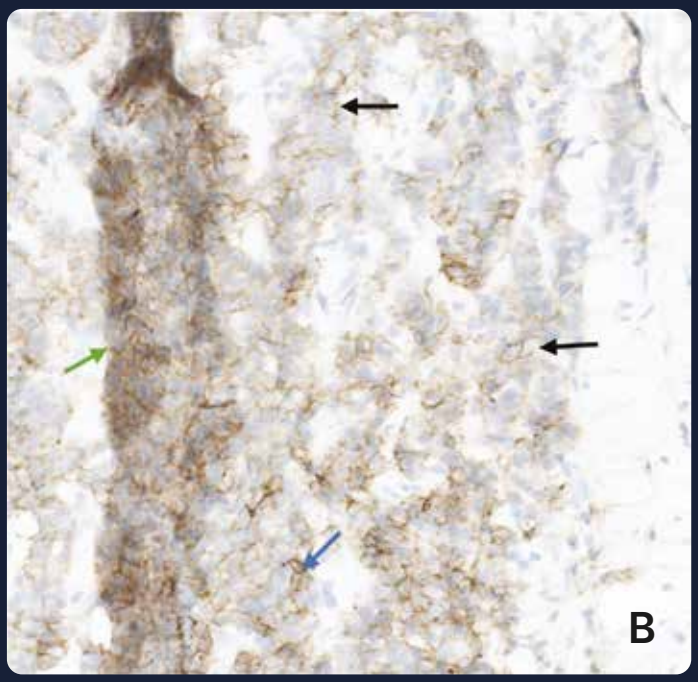

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CASE 23

(A) Moderately to poorly differentiated invasive ductal carcinoma of NST and histologic grade 2. Focal micropapillary features are seen at the edge of one of the tissue cores (not represented here). Tissue folding is identified in multiple areas of the specimen (H&E stained section, 20X magnification).

(B) HER2 IHC assay (20X original magnification). Tumor cells are expressing an incomplete membranous staining of weak to moderate intensity **(black arrows)**. However, some of the tumor cells display a strong and complete membranous staining **(blue arrow)** but, due to the tissue folding artifact **(green arrow)**, it is hard to assess their extent. When put together with the micropapillary features seen on H&E, it would be more appropriate to repeat the staining to ascertain the correct score.

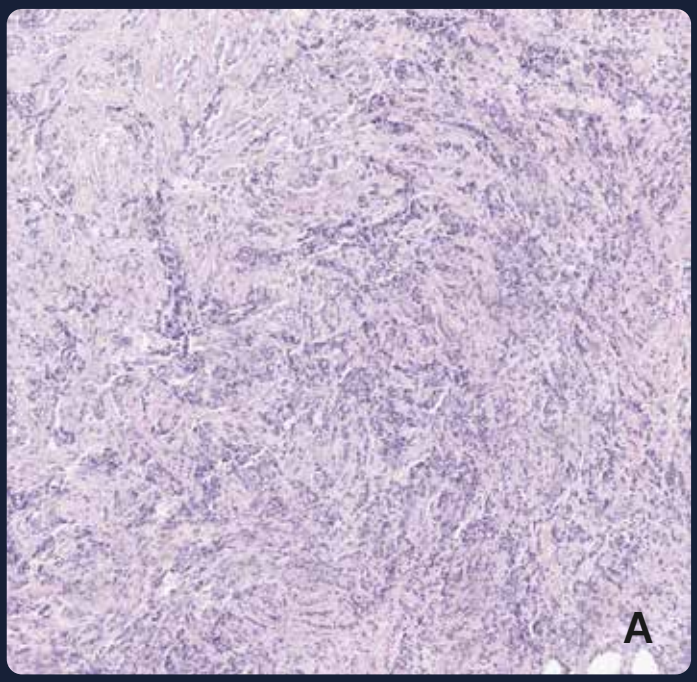
IHC score: indeterminate (cannot be determined)

The use of a different testing method (ISH/FISH) might be indicated if the artifact would persist on the IHC repeat.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Staining patterns in clinical breast cases

Indeterminate (cannot be determined)



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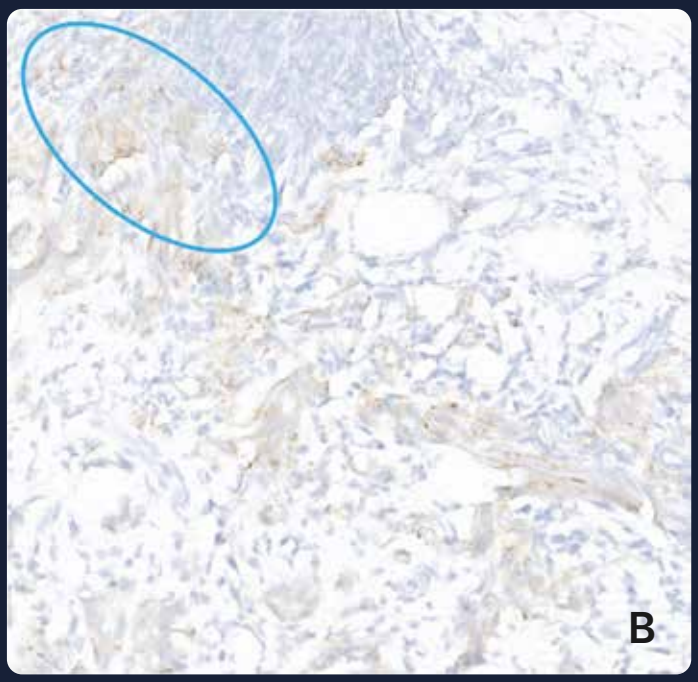

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CASE 24

(A) Moderately differentiated invasive ductal carcinoma with NST and extended crush artifact. Histologic grade 2 (H&E stained section, 5X magnification).

(B) HER2 IHC assay (20X original magnification). Tumor cells display a non-specific cytoplasmic staining in multiple areas where the crush artifact **(blue circle)** had affected the tissue. Only rare preserved tumor cells display a difficult-to-label membranous staining. Due to the extent of the crush artifact and the subsequent suboptimal staining, it would be more appropriate to repeat the stain on a better-preserved tissue section to evaluate the final score.

IHC score: indeterminate (cannot be determined)

The use of a different testing method (ISH/FISH) might be indicated if the artifact would persist on the IHC repeat.

FISH, fluorescent *in situ* hybridization; HER2, human epidermal growth factor receptor 2; H&E, hematoxylin and eosin; IHC, immunohistochemistry; ISH, *in situ* hybridization; NST, no special type.

Conclusion

HER2 is a major prognostic and predictive biomarker in breast cancer.[1] The assessment of HER2 status in breast cancer is critical for disease management and therefore a priority for pathology practice standardization.[2] The commonly used methods for determining HER2 status in breast cancer are IHC and ISH.[3,4] Since the ASCO-CAP guidelines on HER2 testing were first released, they have continued to evolve to provide clear recommendations for HER2 testing in breast cancer.[3]

In spite of the dichotomous interpretation framework, over 50% of breast cancers could be classified as HER2-low, described as IHC 1+ or IHC 2+ with negative ISH (without HER2 amplification).[1,5,6] As clinical evidence continues to grow, it is becoming increasingly important how we, as pathologists, approach differentiating HER2 scores of IHC 0, 1+ and 2+ in a reproducible manner.[7]

In current practice, there is considerable variability in IHC scoring between different laboratories and pathologists, especially on the post-analytical stages of interpreting and reporting of results.[8] Standardization of testing processes promotes quality in HER2 testing, and this should extend to aspects of the testing stages (pre-analytic, analytic and post-analytic).[9] Where deviations from standardized protocols occur, these should be clearly documented in the pathology report.[3]

For patients with breast cancer, a reliable and reproducible diagnostic assessment of HER2 is critical for making optimal treatment decisions.[1,7,8] Pathology practices need to continuously evolve in tandem with clinical advances in the HER2 testing landscape.[10]

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization.

1. Tarantino P, et al. J Clin Oncol 2020;38(17):1951-1962. 2. Hicks DG, Schiffhauer L. Lab Medicine 2011;42:459-467. 3. Wolff AC, et al. J Clin Oncol 2018;36:2105-2122. 4. Furrer D, et al. Am J Clin Pathol 2015;144:686-703. 5. Agostinetto, E, et al. Cancers 2021;13:2824. 6. Pernas S and Tolaney S. Ther Adv Med Oncol 2019;11:1-16. 7. Marchiò C, et al. Semin Cancer Biol 2020;72:123-135. 8. Fernandez AI, et al. JAMA Oncol 2022;8(4):1-4. 9. Pfitzner BM, et al. Modern Pathology 2018;31:607-615. 10. Zhang H and Peng Y. Cancers 2023;15(1):126.

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- View and interact with whole slide images from real breast cancer clinical cases
- Interact with peer-developed clinical support tools, including microscopy aids, reference guides, virtual microscopy sessions, and more
- Test your HER2 IHC scoring against the consensus scoring of our Pathology Faculty with knowledge checking features

Additional information, educational resources, and an extensive HER2 IHC image gallery can be found at **HER2**Know.com

Members of the Breast Pathology Faculty have been contracted as paid advisors through Daiichi Sankyo and AstraZeneca and have received compensation for their time.

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