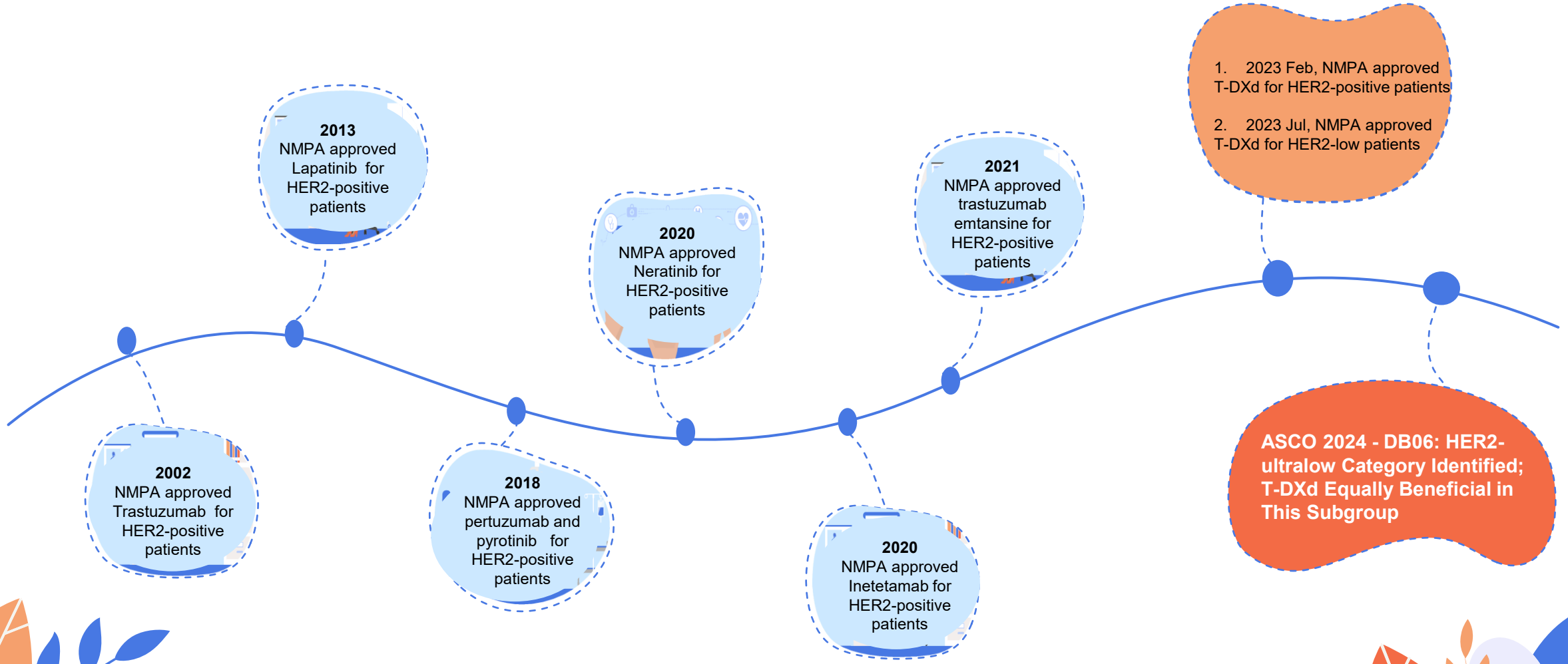
复旦大学附属肿瘤医院
Fudan University Shanghai Cancer Center

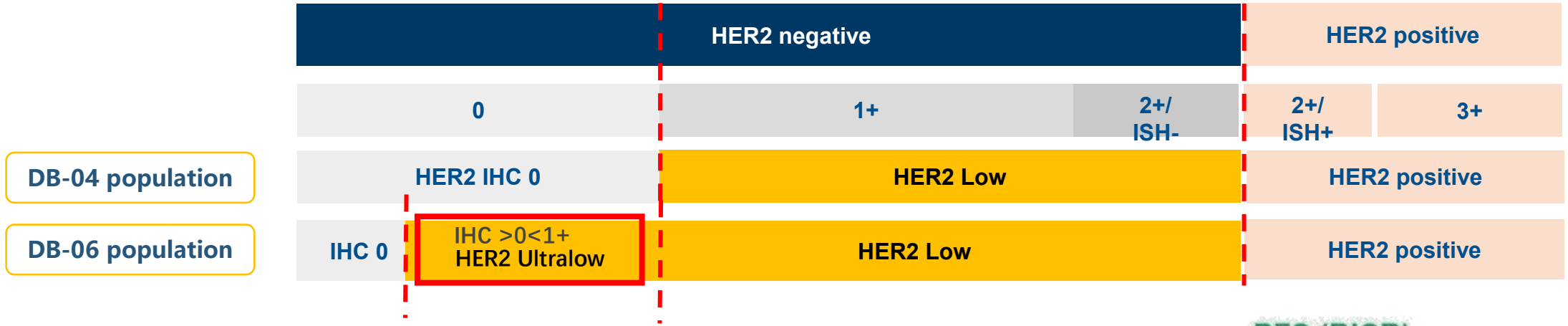
Challenges in HER2 Testing in the Era of HER2-Low and Ultra-Low Breast Cancer

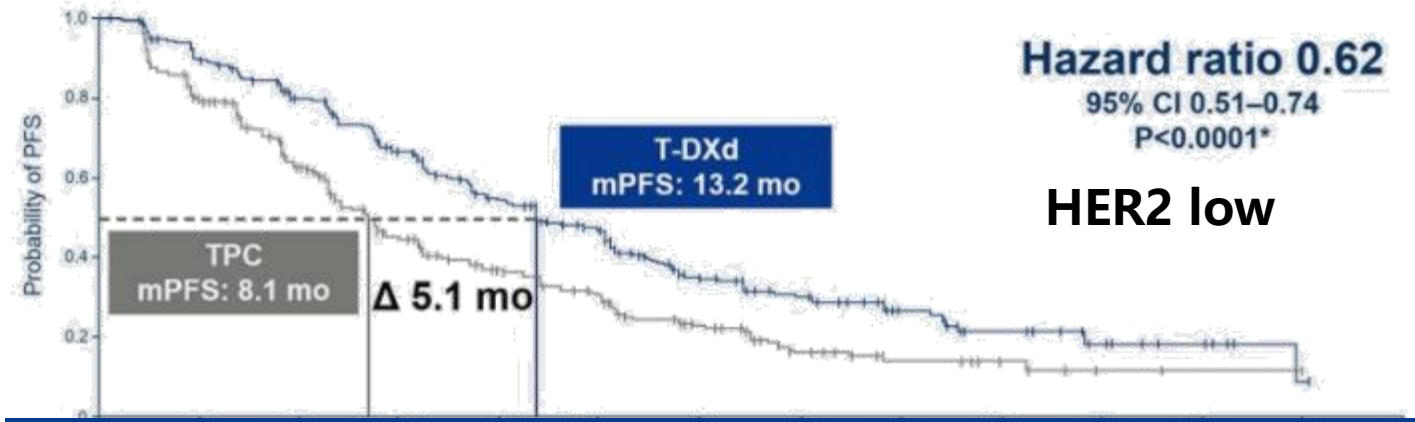
Wentao Yang
Fudan University Shanghai Cancer Center

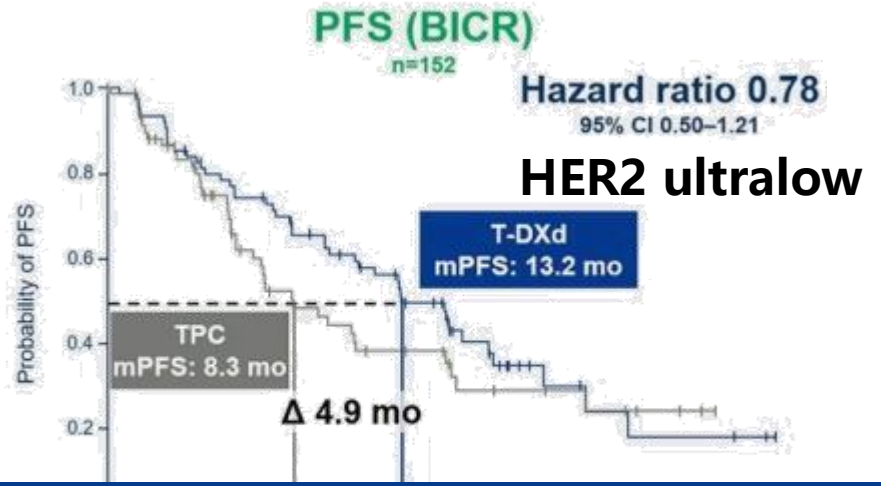
Actionable HER2 Alterations in Breast Cancer have Expanded to Lower Protein Level



DESTINY-Breast04 and DESTINY-Breast06 included patients with HER2 low/ultralow mBC patients



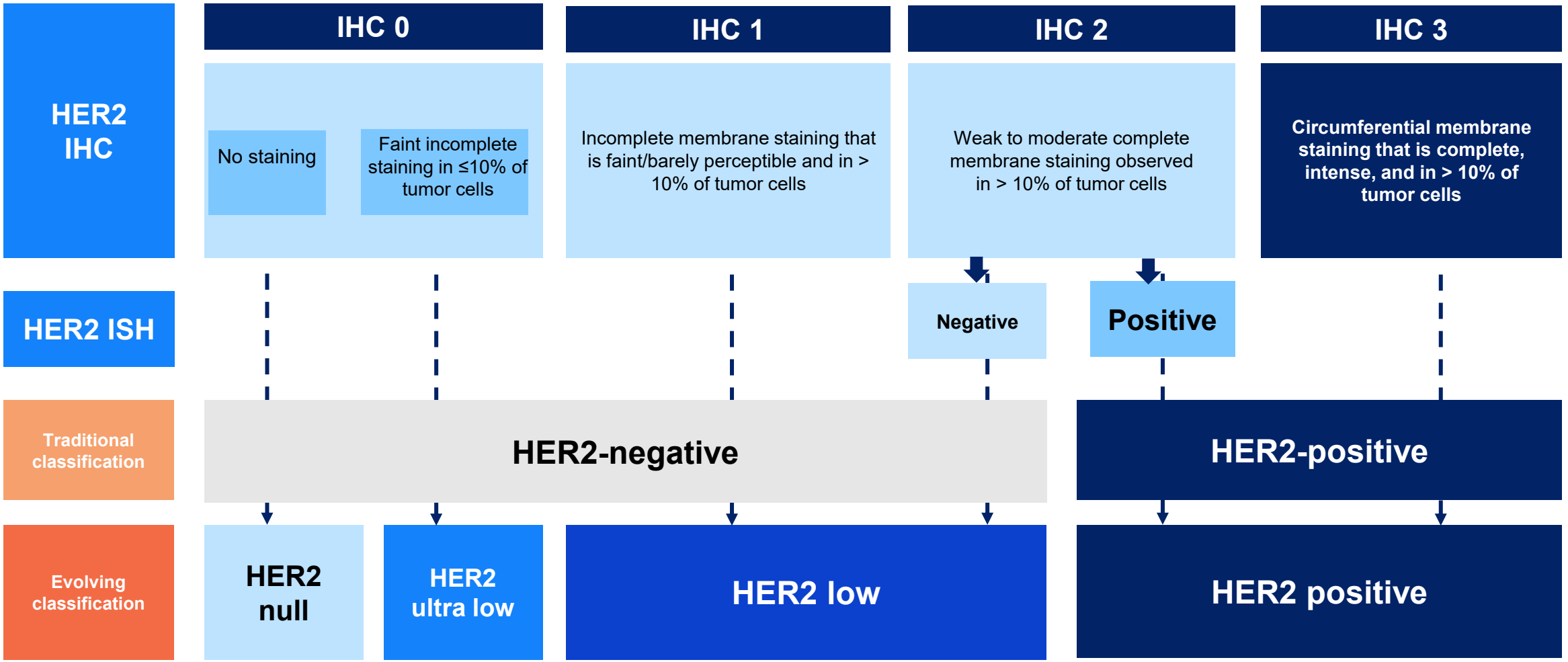




The improved PFS seen in HER2 ultralow patients receiving T-DXd is consistent with the results for HER2 low patients

ClinicalTrials.gov Identifier： NCT04494425

Updating HER2 Testing Landscape



Franchina M, et al. Int J Mol Sci. 2023 Aug 14;24(16):12795

The Evolution of HER2 Testing Guideline in Breast Cancer

Content Points

HER2 testing guideline

First Issue

American Society of Clinical Oncology 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer

2007

Standardize HER2+ testing

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

2013

Refine ISH

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update

2018

FDA Approved HER2 LOW Aug 2022

ESMO HER2 Low Guideline

ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer

Affirmed 2018 ASCO-CAP recommendation include IHC 1+ in footnote

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO–College of American Pathologists Guideline Update

2023

2024

2024 Version Update

乳腺癌HER2检测指南（2024版）

HER2 testing guideline of Breast Cancer in CHINA

2006 **HER2 Testing Guideline (2006)**

2009 **HER2 Testing Guideline (2009)**

2014 **HER2 Testing Guideline (2014)**

2019 **HER2 Testing Guideline (2019)**

T-DXd indication of HER2 low BC approved by CHINA NMPA 2023.07

DB06 Data Readout: HER2 ultralow can benefit from T-DXd

2024 ASCO ANNUAL MEETING

Trastuzumab deruxtecan vs physician's choice of chemotherapy in patients with hormone receptor–positive, human epidermal growth factor receptor 2 (HER2)–low or HER2-ultralow metastatic breast cancer with prior endocrine therapy: primary results from DESTINY-Breast06

2022 Sep 1th Advances and Challenges of HER2 low testing in Breast Cancer

乳腺癌HER2低表达病理检测进展及挑战

Reporting： Both CHINA guideline and CAP recommends to report HER2 ultralow as *IHC 0 with membrane staining*

Table 1 Interpretation algorithm for HER2 immunohistochemistry (IHC)

Category	Final HER2 status		
IHC 3+	>10% of the invasive cancer cells display strong, complete, uniform membrane staining		HER2 positive
IHC 2+	>10% of invasive cancer cells display weak to moderate complete membrane staining, or ≤10% of invasive cancer cells show strong and complete membrane staining	ISH positive	HER2 positive
		ISH negative	HER2 negative (indicating HER2 low)
IHC 1+	>10% of invasive cancer cells display incomplete and faint membrane staining		HER2 negative (indicating HER2-low)
IHC 0	No staining or ≤10% of invasive cancer cells display incomplete and faint membrane staining	With membrane staining	HER2 negative (indicating HER2-ultralow)
		Without membrane staining	HER2 negative (indicating HER2 null)

HER2, human epidermal growth factor receptor 2; ISH, in situ hybridisation.

___ HER2 by Immunohistochemistry (IHC) Status

HER2 by Immunohistochemistry (IHC) Status (Note C)

 # Breast cancers with HER2 IHC scores of 0+, 1+, or 2+ (ISH negative) may be eligible for treatment targeting non-amplified levels of HER2 expression in the metastatic setting. Currently, patients with no membrane staining by IHC (0) are ineligible / excluded. Consider using the optional standardized HER2 IHC report comment to explain the clinical relevance of lower levels of HER2 IHC staining in the metastatic setting and definitions of "ultralow and low" HER2 used in clinical trials. See Note C.

___ Negative (Score 0)#

___ No membrane staining detected (0 / absent membrane staining)

___ Membrane staining that is incomplete and is faint / barely perceptible and in less than or equal to 10% of tumor cells (0+ / with membrane staining)

___ Other (specify): _____

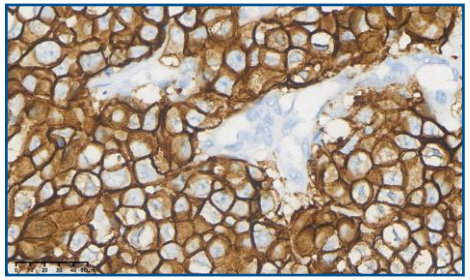
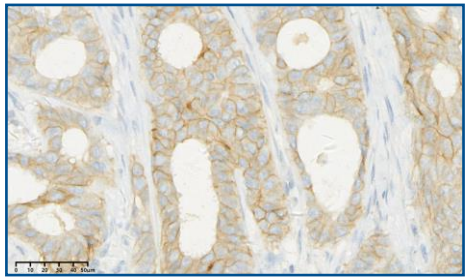
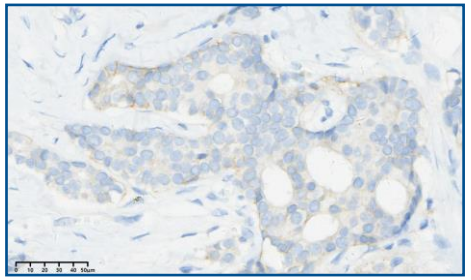
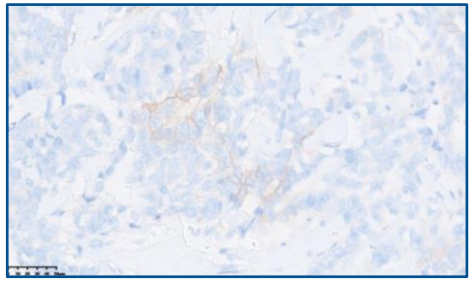
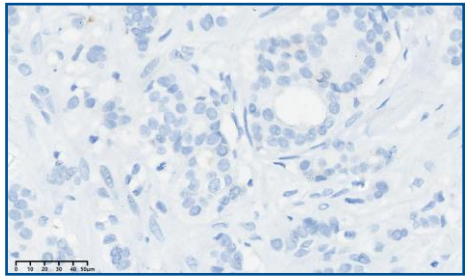
Liu Y, et al. J Clin Pathol 2025;0:1–8. doi:10.1136/jcp-2025-210175

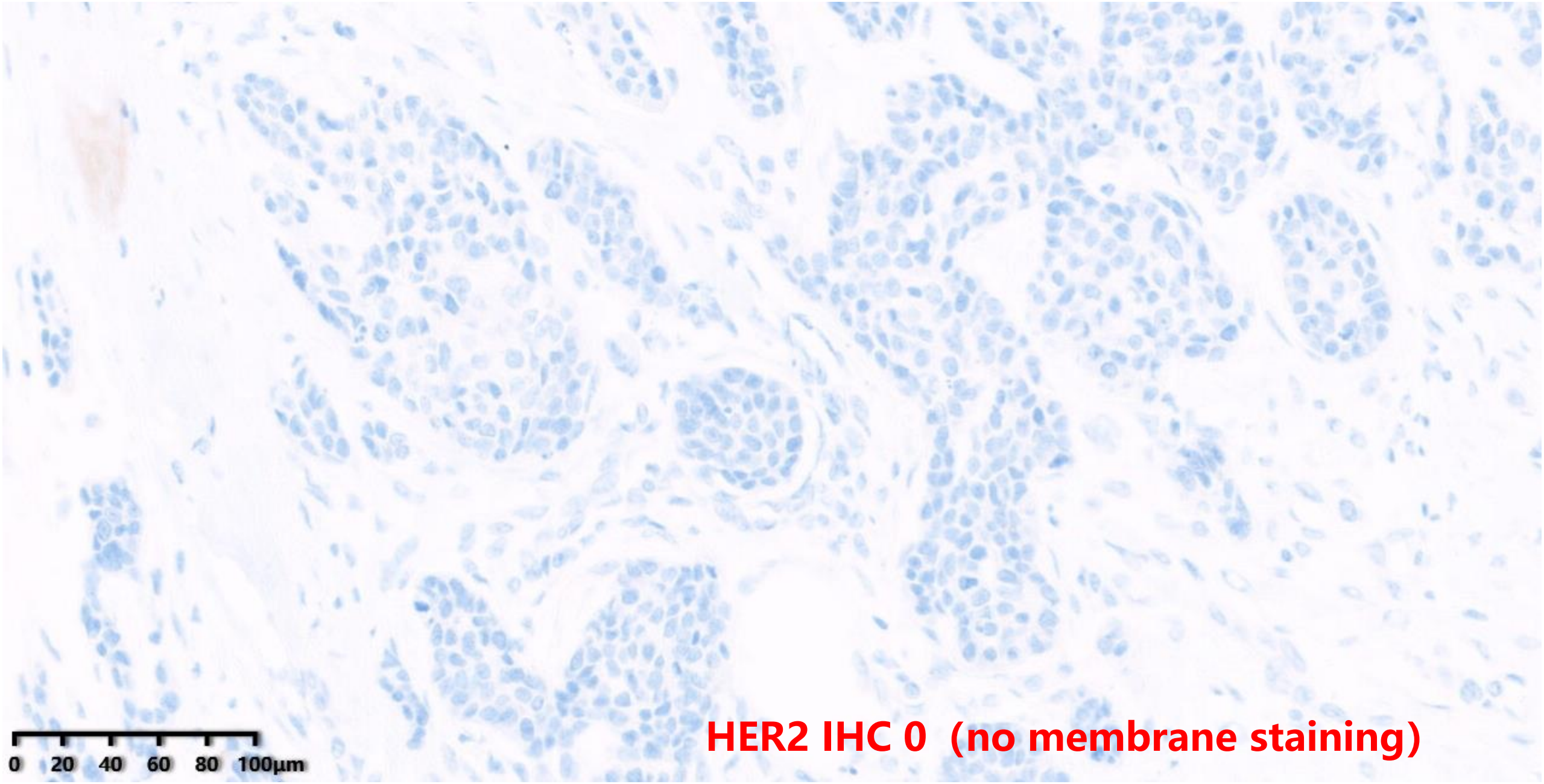
CAP Reporting Template for Reporting Results of Biomarker Testing of Specimens from Patients with Carcinoma of the Breast. Version: 1.6.0.0

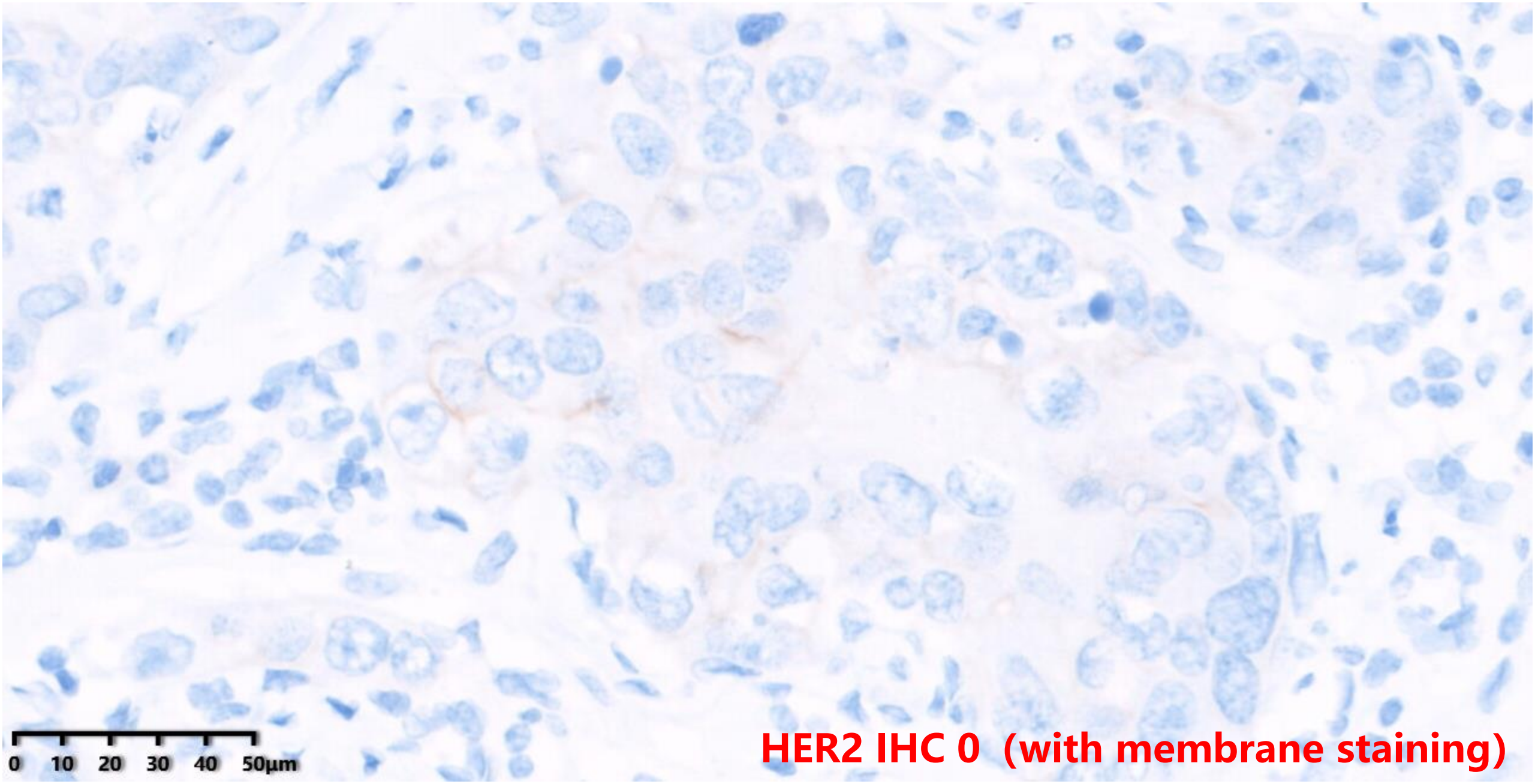
Interpretation Standard for HER2 IHC assays including HER2 ultralow

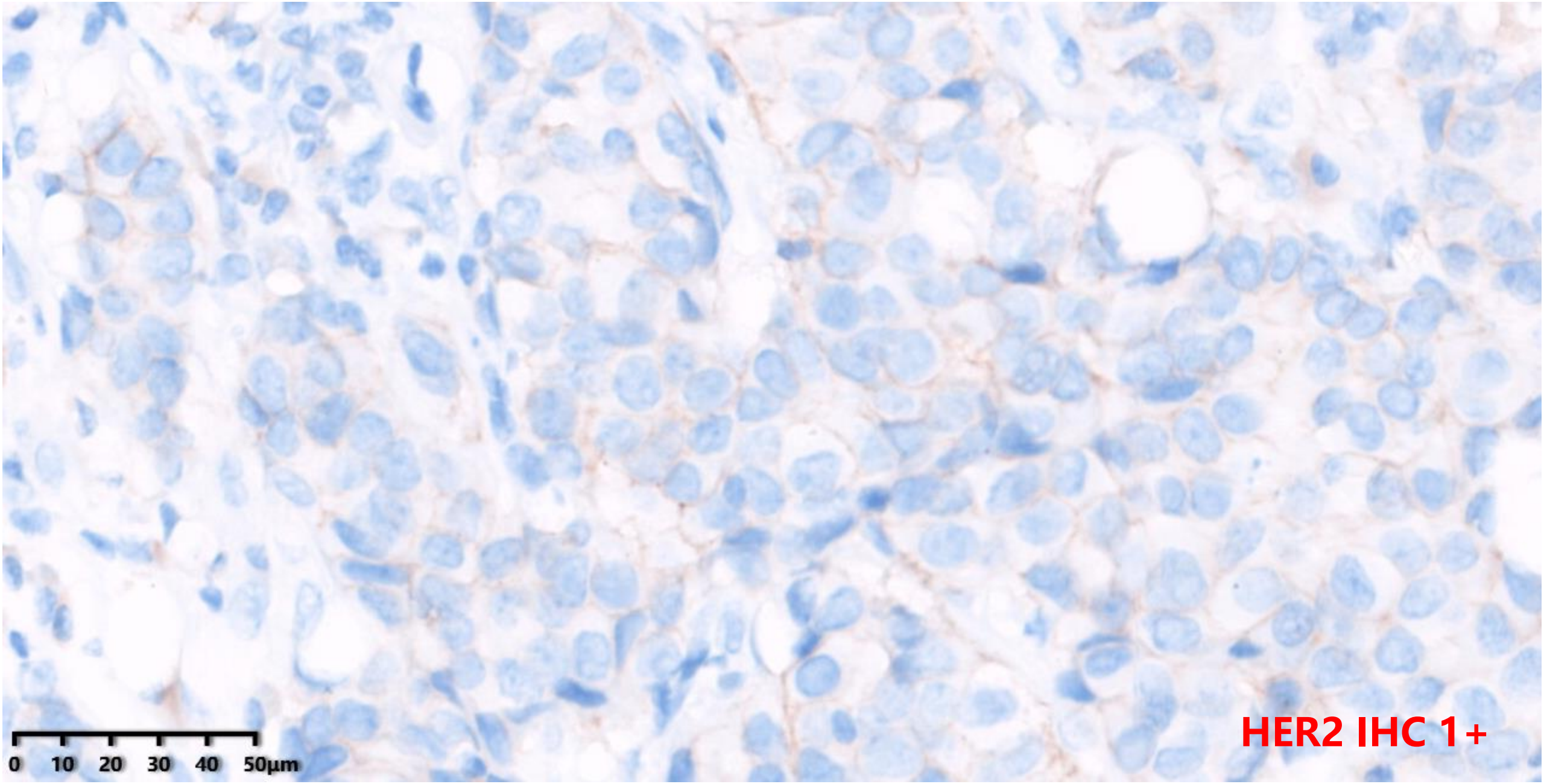
HER2 testing by validated IHC assays

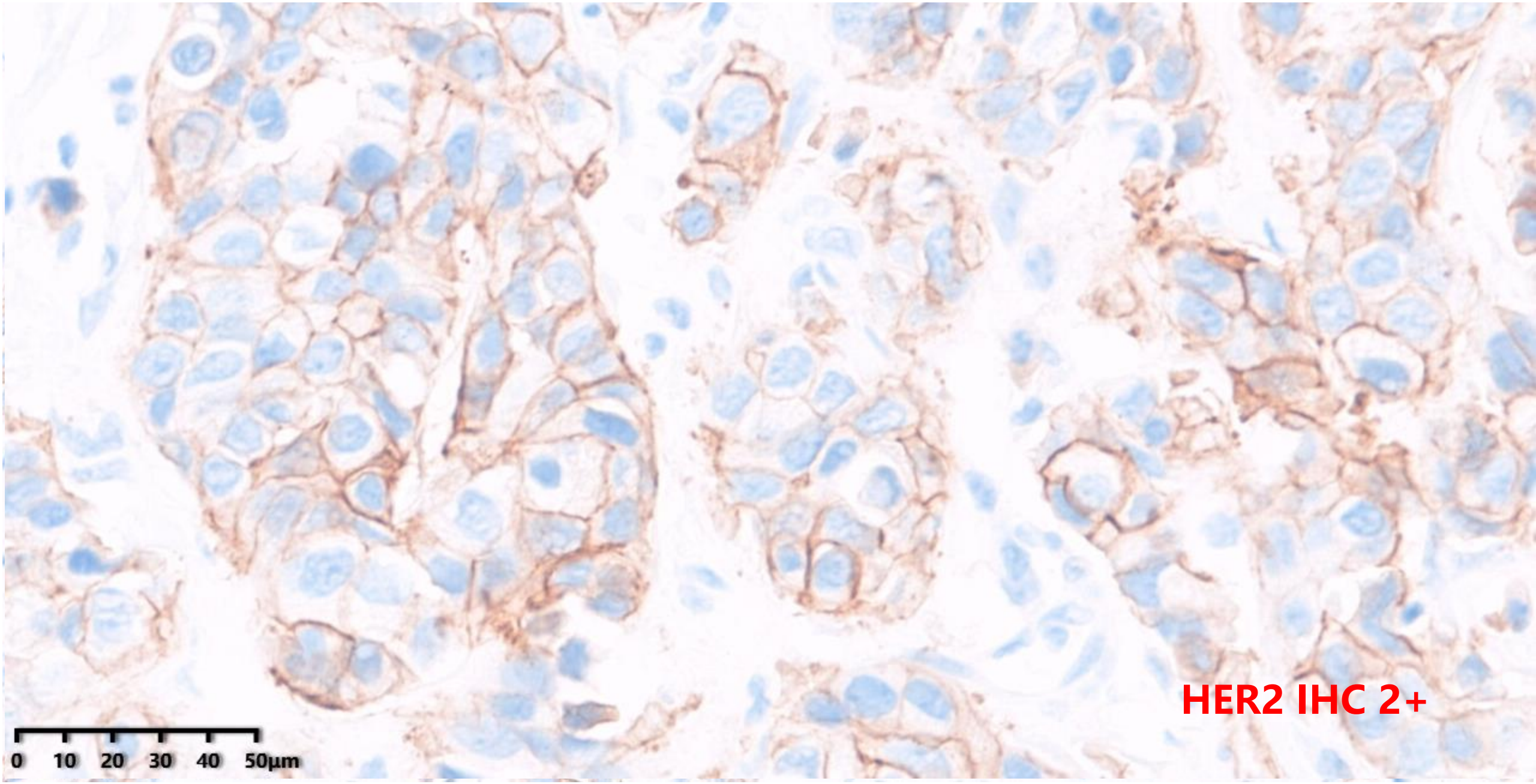
controls show appropriate staining

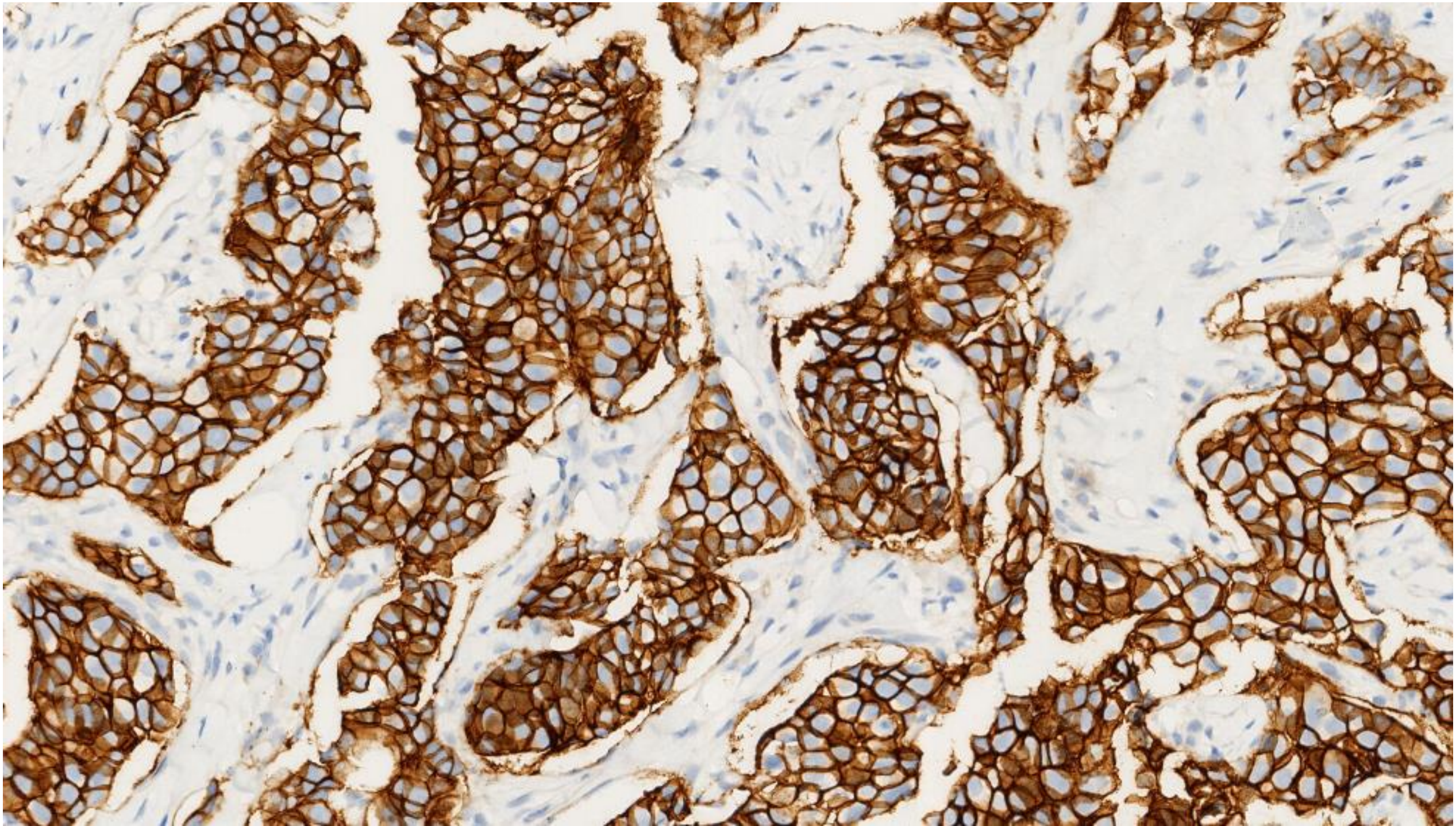
>10% of the invasive cancer cells exhibit strong, complete, uniform membrane staining	> 10% of invasive cancer cells exhibit weak to moderate, complete membrane staining, or ≤10% invasive cancer cells exhibit strong, complete membrane staining	>l0% of invasive cancer cells exhibit incomplete, faint membrane staining	invasive cancer cells with no staining or ≤l0% exhibit incomplete, faint membrane staining	
IHC 3+ Positive	IHC 2+ equivocal	IHC 1+	**IHC 0（with membrane staining）**	**IHC 0（no staining）**
				


0 20 40 60 80 100µm
HER2 IHC 0 (no membrane staining)


0 10 20 30 40 50μm
HER2 IHC 0 (with membrane staining)


0 10 20 30 40 50μm
HER2 IHC 1+

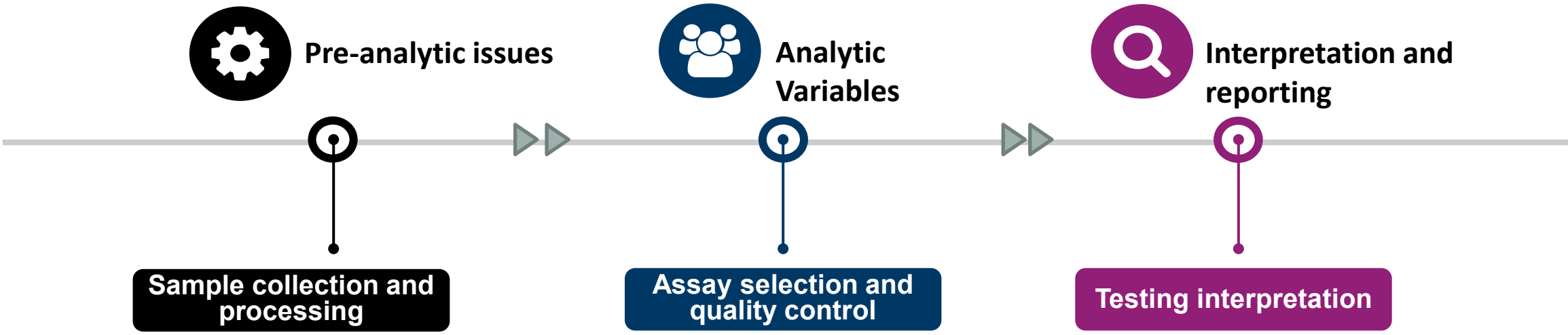

HER2 IHC 2+
0 10 20 30 40 50μm



What Challenges do Pathologists Face Regarding HER2 low/ultra-low?

Pre-analytic and Analytic Variables can Affect the Accuracy and Reproducibility of HER2 Testing

For HER2 ultralow, attention also needs to be paid to the whole process



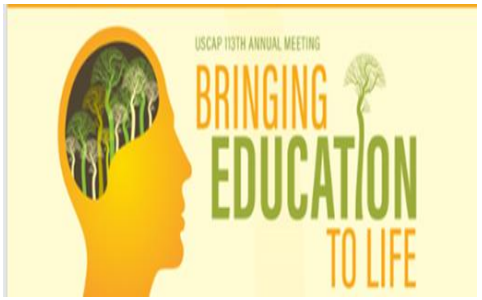
- Sample type-spatial heterogeneity
- Fresh sample vs archived sample
- Cold ischemic time
- Tissue fixation
- Decalcification process
- Preservation of unstained slides and paraffin blocks

- Different types of antibodies and platforms
- Different staining parameters
- Standard Operating Procedure (SOP)
- Negative and positive control

- Pathologist's interpretation ability
- Report presentation format

- Narayan P, et al. J Clin Oncol. 2023.

Pre-analytical： Prolonged cold ischemic time can result in a reduction of HER2 expression



259 Quantitative Assessment of the Effect of Cold Ischemic Time on Immunohistochemical Evaluation of HER2 Expression in Invasive Breast Carcinoma

Lirui Yang[1], Ping Zhu[2], Ming Li[1], Hong Lv[1], Mingzhen Lin[3], Xu Cai[1], Wenhua Jiang[4], Wentao Yang[1], Bao-Hua Yu[1]

[1]Fudan University Shanghai Cancer Center, Shanghai, China, [2]Fudan University Shanghai Cancer Center, Fudan University, Shanghai, China, [3]HangZhou DiYingJia Technology Co.,Ltd., Hangzhou, China, [4]Fudan University Shanghai Cancer Center, Shanghai Medical College, Fudan University, Shanghai, China

Prospective study: Collect cases of invasive breast cancer from Fudan University Shanghai Cancer Center

↓

The mass was divided into six tissue blocks with a size of 5mm * 5mm * 3mm, and placed in 10% NBF according to the following time

↓

0H | 1H | 2H | 4H | 8H | 24H

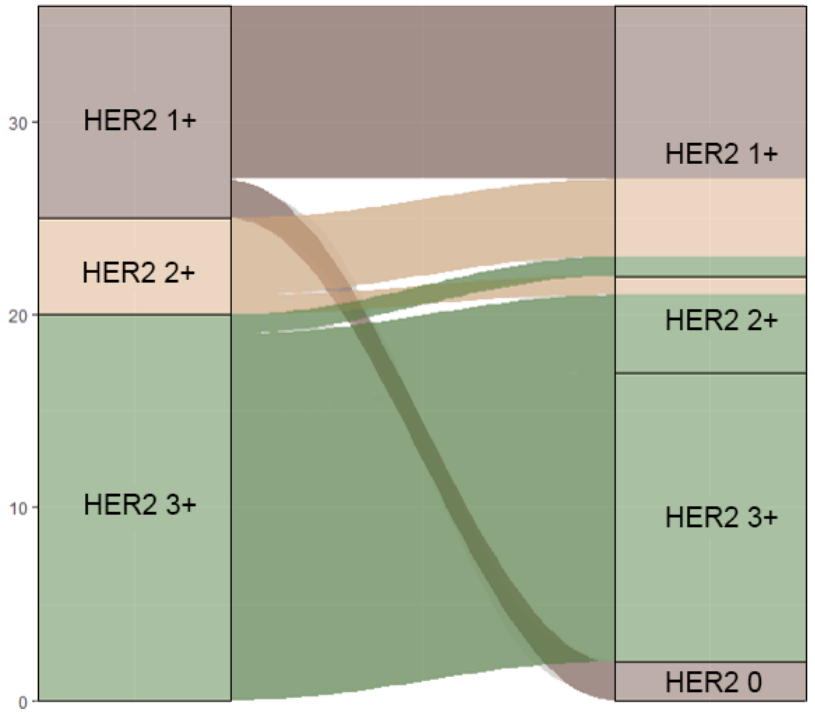
Note: All tissue blocks after separation are stored at room temperature (22 ℃-26 ℃)

↓

IHC staining is performed after fixation for 24-48 hours

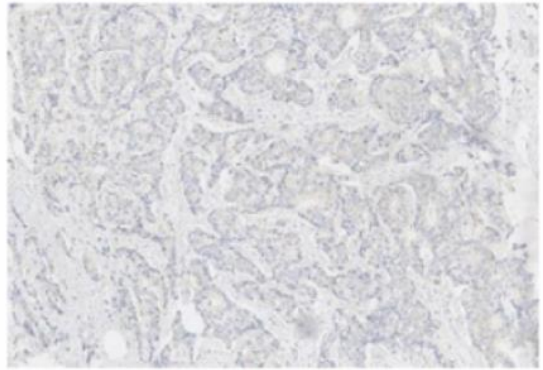
↓

AI evaluated the influence of different cold ischemic times on the HER2 IHC results of the same tumor tissues, especially the influence on patients with HER2 low expression



- Compared with timely fixation (0.5 hours after resection), there is a notable decline in the percentage of membrane staining in samples with CIT of 4 hours, 8 hours and 24 hours.
- Delayed fixation has an adverse effect on HER2 expression, especially for cases with low HER2 expression

Lirui Yang, Baohua Yu, et al. 2024 USCAP Breast Pathology Abstract 259

Day 0 Staining	Stability of the unstained slides  PATHWAY HER2 (4B5)			
	Low T/Low H	**Low H/High T**	**High T/Low H**	**High T/High H**
Time Point	5°C±3°C RH 15%±10%	5°C±3°C RH 85%±10%	30°C±5°C RH 15%±10%	30°C±5°C RH 85%±10%
Day 7	12/12 (100%)	8/8 (100%)	8/8 (100%)	6/12 (50%)
Day 45	12/12 (100%)	8/8 (100%)	8/8 (100%)	4/12 (33%)
Month 2	12/12 (100%)	8/8 (100%)	8/8 (100%)	2/12 (17%)
Month 3	12/12 (100%)	8/8 (100%)	8/8 (100%)	2/8 (25%)
Month 4	12/12 (100%)	8/8 (100%)	8/8 (100%)	2/6 (33%)
Month 6	12/12 (100%)	6/8 (75%)	2/8 (25%)	2/6 (25%)
Month 7	8/12 (67%)	4/8 (50%)	2/8 (25%)	2/4 (50%)
Month 9	6/12 (50%)	Not assessed	Not assessed	Not assessed

- High temperature（30°C±5°C）/high humidity（85% ± 10%）:staining lost at day 7.
- High temperrature（30°C±5°C）/low humidity（15% ± 10%）:staining lost at month 6.
- Low temperature（5°C±3°C）/high humidity（85% ± 10%）:staining lost at month 6.
- Low temperature （5°C±3°C） / low humidity （15% ± 10%）: staining lost a month 7.

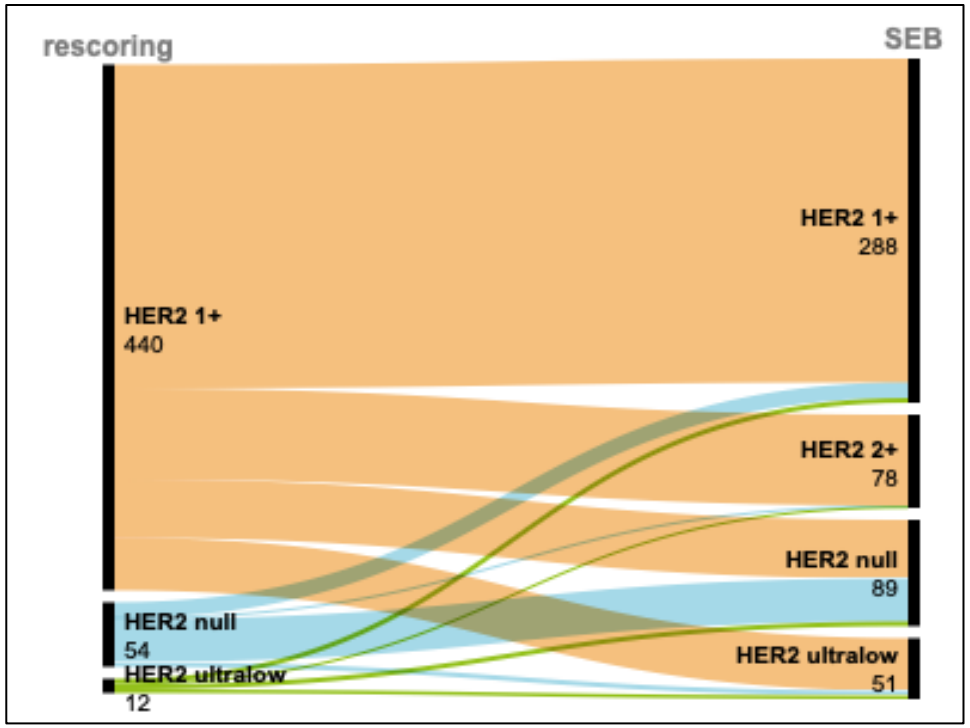
Virchows Arch. 2024 Jun;484(6):1005-1014

Pre-analytical: Heterogeneity Existed Between CNB and Resection Samples

HER2 ultra-low status is also different

Artificial Intelligence Assisted Assessment of HER2-ultralow and HER2-low Immunohistochemical Scoring in Breast Cancer Core Needle Biopsy Specimens

PRESENTED BY
Ming Li
Fudan University Shanghai Cancer Center, Shanghai, China



- The overall discordance rate of HER2 status between core needle biopsy (CNB) and subsequent excision biopsy (SEB) samples was 22.13%.
- 28.79% (19/66) of initially HER2 null/ultra-low cases converting to HER2-low in SEB.
- 21.14% (93/440) of initially HER2-low cases reverting to HER2 null/ultra-low in SEB.
- These transitions may reflect underlying intratumoral heterogeneity.

Ming Li, Wentao Yang, et al. 2024 USCAP Breast Pathology Abstract 178

Pre-analytical： Different Paraffin Blocks can Show Different HER2 Low/ultra-low Status

181 **Analysis of the Impact of Varied Tumor FFPE Blocks on the Diagnosis of HER2 IHC 0, Ultra-low and 1+ in Breast Cancer**

Hongbo Liu[1], Si Wu[1], Yueping Liu[1]
[1]*The Fourth Hospital of Hebei Medical University, Shijiazhuang, China*

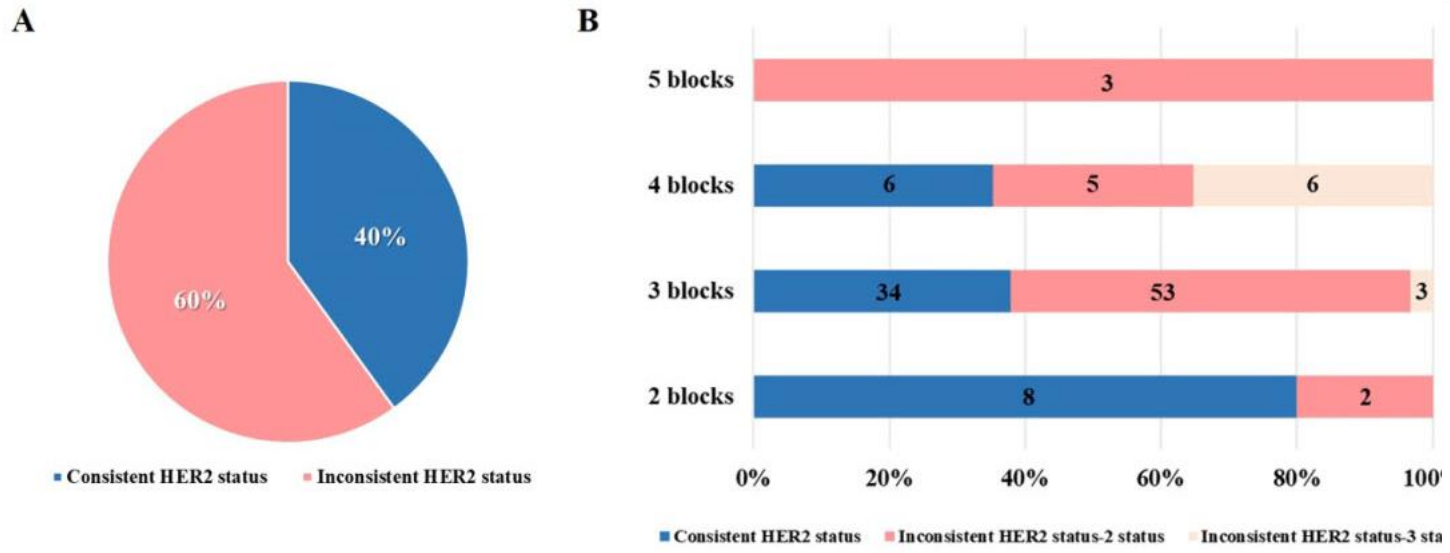


Figure 1. Inconsistency in HER2 results of overall and with different numbers of FFPE blocks

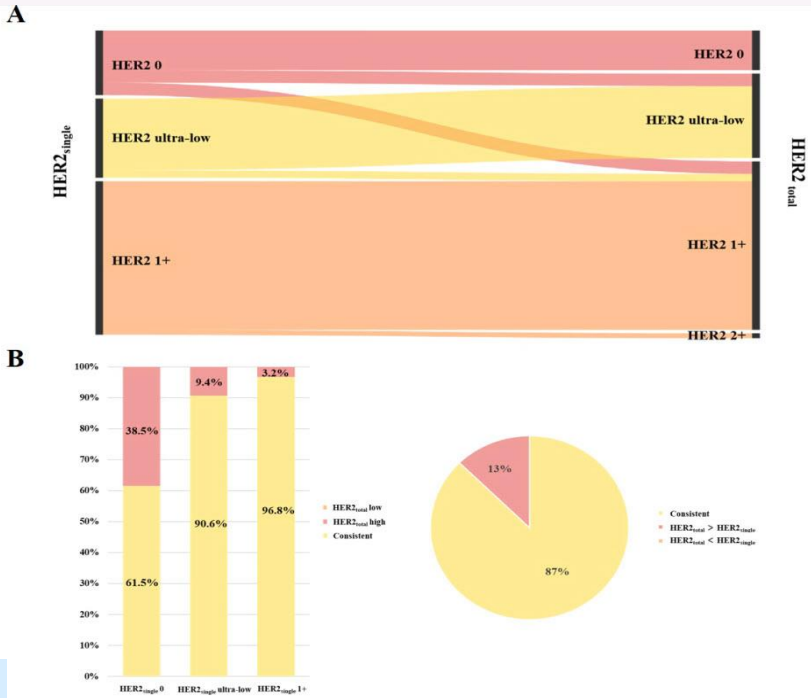
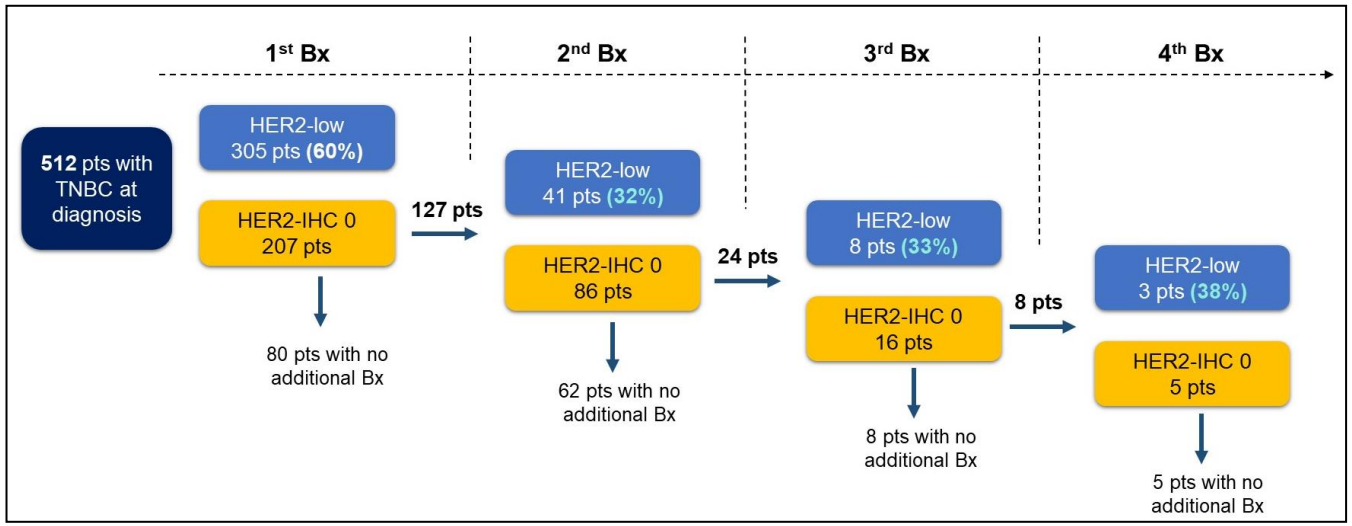


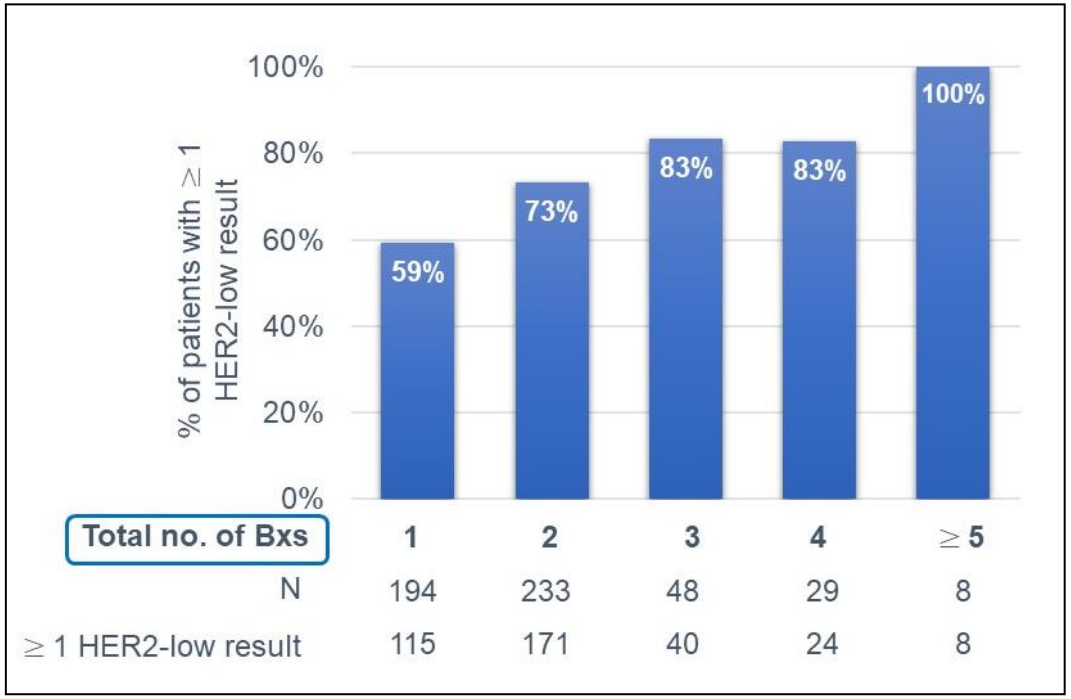
Figure 2. Inconsistency in HER2 results between single FFPE block and multi-FFPE blocks

- In 120 cases, 13.3% were HER2 0 and 28.3% were HER2 ultra-low. There was a 60.0% inconsistency in HER2 scores among different FFPE blocks (Figure 1A). Among them, 62.2% had inconsistent HER2 scores among three FFPE blocks, and 64.7% were inconsistent among four FFPE blocks (Figure 1B).
- Multi-FFPE block detection will increase the number of patients who may benefit from T-DXd treatment by 10 cases.

Pre-analytical： Will HER2 Low be Rediscovered by Repeated Biopsies for HER2 0 Cases



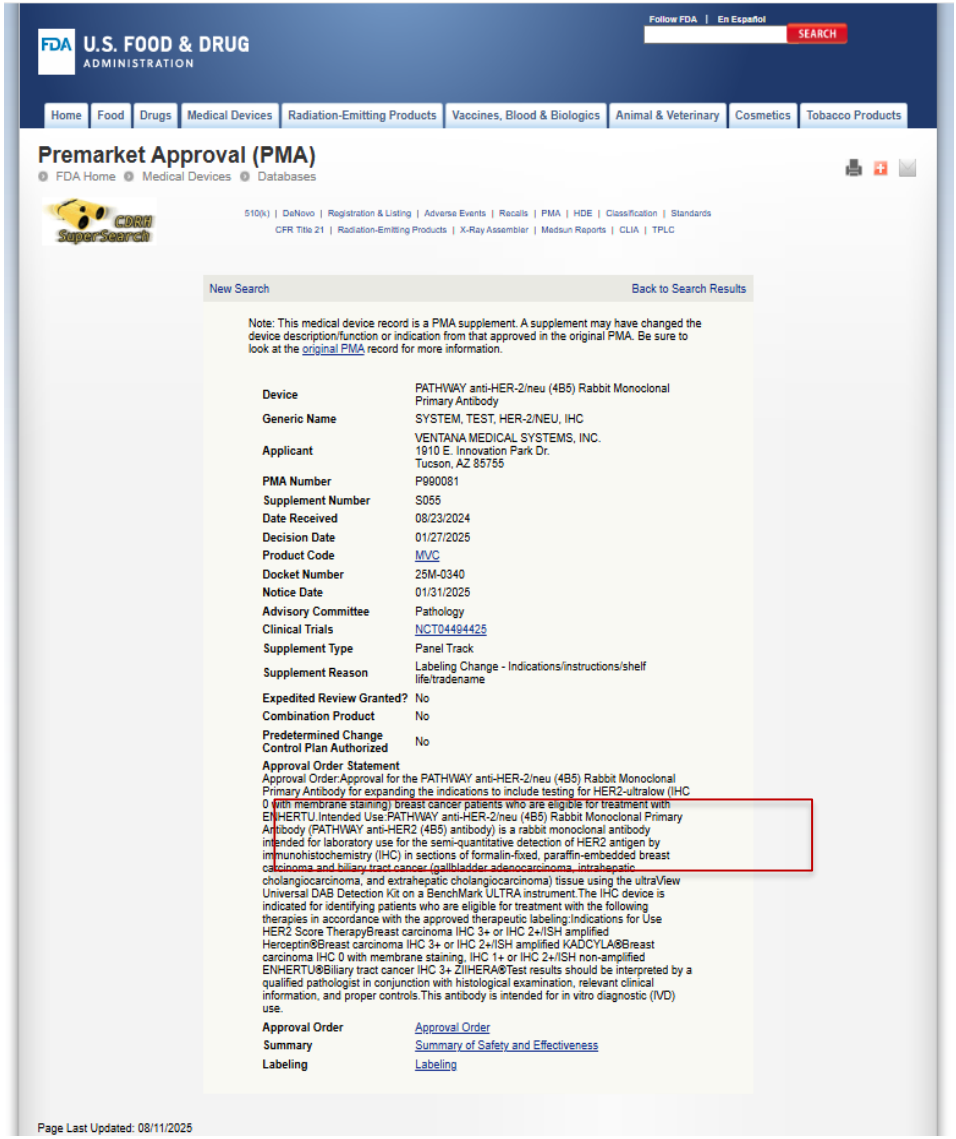
In breast cancer patients without a prior HER2-low result, about one third converted to HER2-low with each successive additional biopsy



- Repeat biopsy should be considered for patients with prior HER2 0 results, as it may reveal HER2-low status and impact treatment decisions.
  Re-testing strategies, including re-biopsy or sample re-analysis, could identify eligible patients for novel ADC therapies among those initially deemed HER2-zero.
- The clinical significance of such HER2 dynamics—whether biological or technical—warrants validation.

Yael Bar et al. JCO 41, 1005-1005(2023).

Analytical：Roche 4B5 assay is approved by FDA as HER2 IHC CDx assay for HER2-ultralow assessing



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Note: This medical device record is a PMA supplement. A supplement may have changed the device description/function or indication from that approved in the original PMA. Be sure to look at the original PMA record for more information.

Device	PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody
Generic Name	SYSTEM, TEST, HER-2/NEU, IHC
Applicant	VENTANA MEDICAL SYSTEMS, INC. 1910 E. Innovation Park Dr. Tucson, AZ 85755
PMA Number	P990081
Supplement Number	S055
Date Received	08/23/2024
Decision Date	01/27/2025
Product Code	MVC
Docket Number	25M-0340
Notice Date	01/31/2025
Advisory Committee	Pathology
Clinical Trials	NCT04494425
Supplement Type	Panel Track
Supplement Reason	Labeling Change - Indications/instructions/shelf life/tradename
Expedited Review Granted?	No
Combination Product	No
Predetermined Change Control Plan Authorized	No

Approval Order Statement

Approval Order:Approval for the PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody for expanding the indications to include testing for HER2-ultralow (IHC 0 with membrane staining) breast cancer patients who are eligible for treatment with ENHERTU.Intended Use:PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody (PATHWAY anti-HER2 (4B5) antibody) is a rabbit monoclonal antibody intended for laboratory use for the semi-quantitative detection of HER2 antigen by immunohistochemistry (IHC) in sections of formalin-fixed, paraffin-embedded breast carcinoma and biliary tract cancer (gallbladder adenocarcinoma, intrahepatic cholangiocarcinoma, and extrahepatic cholangiocarcinoma) tissue using the ultraView Universal DAB Detection Kit on a BenchMark ULTRA instrument.The IHC device is indicated for identifying patients who are eligible for treatment with the following therapies in accordance with the approved therapeutic labeling:Indications for Use HER2 Score TherapyBreast carcinoma IHC 3+ or IHC 2+/ISH amplified Herceptin®Breast carcinoma IHC 3+ or IHC 2+/ISH amplified KADCYLA®Breast carcinoma IHC 0 with membrane staining, IHC 1+ or IHC 2+/ISH non-amplified ENHERTU®Biliary tract cancer IHC 3+ ZIIHERA®Test results should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.This antibody is intended for in vitro diagnostic (IVD) use.

Approval Order	Approval Order
Summary	Summary of Safety and Effectiveness
Labeling	Labeling

Page Last Updated: 08/11/2025

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

ORIGINAL ARTICLE

Analytical and clinical validation of PATHWAY HER2 (4B5) assay for assessment of HER2-low/HER2-ultralow status and eligibility for trastuzumab deruxtecan in DESTINY-Breast06

R. Shami[1*], R. Salgado[2,3], A. Bardia[4], G. Curigliano[5,6], X. Hu[7,8], R. Dent[9], J.-Y. Pierga[10], J. Tsurutani[11], H. Wildiers[12], G. Ricciardi[13], C. Marchiò[14,15], F. Penault-Llorca[16], C. Bor-Angelier[17], M. Manoogian[18*], S. Lucas[18], M. T. Olson[18], X. Liu[18], P. Toro[19], A. F. Baker[18], Q. Fang[18], J. Su[18], A. Yoder[18], A. Andrzejuk-Ćwik[20], A. Darilay[21], T. Matsuo[22], F. Jones[1] & G. Viale[23]

Table 1. Inter-laboratory reproducibility of the Roche HER2 4B5 assay on BC tissue with HER2-ultralow scoring on BenchMark ULTRA

Inter-laboratory reproducibility (n = 28)	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	413/420	98.3	96.7-99.8
	NPA	420/420	100	99.1-100
Within-site	PPA	413/420	98.3	96.7-99.8
	NPA	420/420	100	99.1-100
Within-reader	PPA	413/420	98.3	96.7-99.8
	NPA	420/420	100	99.1-100
Between-site	APA	8124/8260	98.4	96.7-99.8
	ANA	8404/8540	98.4	96.9-99.8
Between-reader	APA	406/413	98.3	96.6-99.8
	ANA	420/427	98.4	96.8-99.8
Between-day	APA	1628/1652	98.5	97.2-99.8
	ANA	1684/1708	98.6	97.4-99.8

ANA, average negative agreement; APA, average positive agreement; BC, breast cancer; CI, confidence interval; HER2, human epidermal growth factor receptor 2; NPA, negative percent agreement; PPA, positive percent agreement.

The CDRH review team believes that the analytical validation data and clinical performance data for the PATHWAY anti-HER2/neu (4B5) rabbit monoclonal primary antibody test support its use as a companion diagnostic for screening patients with breast cancer who have ultra-low HER2 expression.

1. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P990081S055; 2. Shami R, et al. ESMO Open. 2025;10(6):105310. doi:10.1016/j.esmoop.2025.105310

Analytical

Concordance Between the DESTINY-Breast04 Clinical Trial Assay (4B5[CDx]) and Other HER2 IHC Assays for HER2-low Breast Cancer in Real-World Practice: First Phase of a Large-Scale, Multicenter Global Ring Study

- **39 laboratories** from the United States, Canada, and Europe without using 4B5(CDx), and **76 pathologists** participated in the first phase of the study.
- 50 BC samples were chosen by a steering committee composed of expert pathologists, and stained in a central laboratory using 4B5(CDx), 15 samples were scored as 0, 17 as 1+, 13 as 2+, and 5 as 3+.
- The unstained sections of the selected cases were then sent to the comparator laboratories.
- Pathologists receive virtual guideline alignment focusing on HER2 low identification after they completed the baseline scoring in 14 days, then re-score the samples after 2 week washout period
- The pre- and post-alignment scores are centrally analyzed to determine the consistency with 4B5(CDx) in identifying HER2 low expression cases

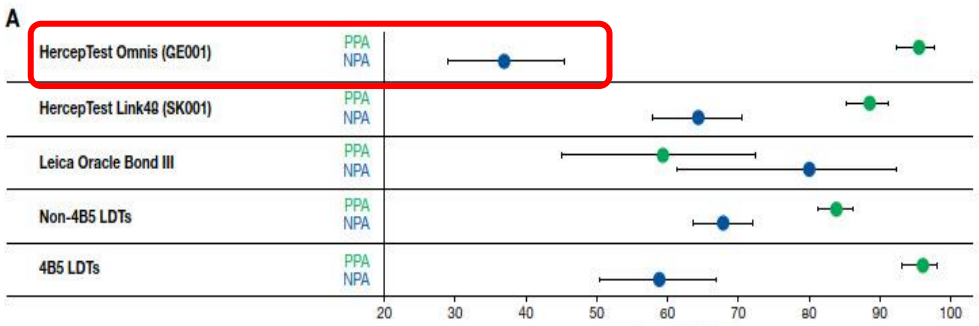
Table 3. HER2-low Versus HER2 IHC 0 Postalignment Assessments

		HER2-low vs HER2 IHC 0 BC Postalignment Scores	
		PPA (95% CI), %	NPA (95% CI), %
Overall postalignment CA test results (N = 3017)		87.5 (86.0-89.0)	61.9 (58.9-64.9)
CA subgroups	HercepTest Omnis (GE001) n = 408	95.5 (92.3-97.7)	36.9 (28.9-45.4)
	HercepTest Link48 (SK001) n = 707	88.5 (85.2-91.2)	64.3 (57.8-70.4)
	Leica Oracle Bond III n = 84	59.3 (45.0-72.4)	80.0 (61.4-92.3)
	Non-4B5 LDTs n = 1395	83.8 (81.2-86.1)	67.8 (63.5-72.0)
	4B5 LDTs n = 423	96.0 (93.0-98.0)	58.8 (50.4-66.8)

Table 4. Postalignment AUROC and Cohen κ Scores for HER2-low versus HER2 IHC 0

		HER2-low vs HER2 IHC 0 BC	
		AUROC[a]	Cohen κ (95% CI)
Overall		0.77	0.51 (0.48-0.54)
CA subgroups	HercepTest Omnis (GE001)	0.78	0.37 (0.28-0.46)
	HercepTest Link48 (SK001)	0.78	0.55 (0.48-0.61)
	Leica Oracle Bond III	0.68	0.35 (0.17-0.53)
	Non-4B5 LDTs	0.76	0.52 (0.47-0.57)
	4B5 LDTs	0.85	0.59 (0.51-0.68)

Figure 1. Forest Plots for Postalignment PPA and NPA for (A) CA and (B) Country Subgroups



Concordance Across Subgroups

- PPA tended to be high across all subgroups
- Postalignment NPA tended to be lower across subgroups
- NPA tended to show more variability between assay types

Concordance Between the 4B5(CDx) and CAs

- The postalignment PPA and NPA for the overall scores were 87.5% and 61.9%, respectively. Highest PPA seen with 4B5 LDTs (96%)
- The Cohen κ value for the comparison of the overall CA postalignment scores with the 4B5(CDx) scores was 0.51

The highest Cohen κ value was seen with 4B5 LDTs (κ = 0.59)

- The area under the receiver operating characteristic curve (AUROC) was generally between 0.7 and 0.8 for most subgroups. The AUROC showed agreement above 0.8 for the 4B5 LDTs

Concordance between the 4B5(CDx) and CAs in the ability to categorize HER2-low versus HER2 IHC 0 varied among assay types

Hans-Ulrich Schildhaus, Sunil Badve, Corrado D Arrigo, et al. AACR; Cancer Res 2024;84(6_Suppl) Abstract nr 1030.

Analytical：Will different Antibodies or Platforms Affect Result of HER2 Ultra-low?

Most HER2 antibodies are developed and verified for HER2 positive. For the recognition of weak staining signals such as HER2 low and HER2 ultra-low, further verification is needed.

Hercep Test (mAb) detected more HER2-low cases compared with Ventana 4B5

Table 1 Comparison of HER2 scorings derived from the indicated IHC assays

		PATHWAY 4B5				
		0	1+	2+	3+	Total
Hercep Test (mAb)	0	35	0	0	0	35
	1+	17	8	0	0	25
	2+	4	12	13	1	30
	3+	0	0	2	27	29
	Total	56	20	15	28	119

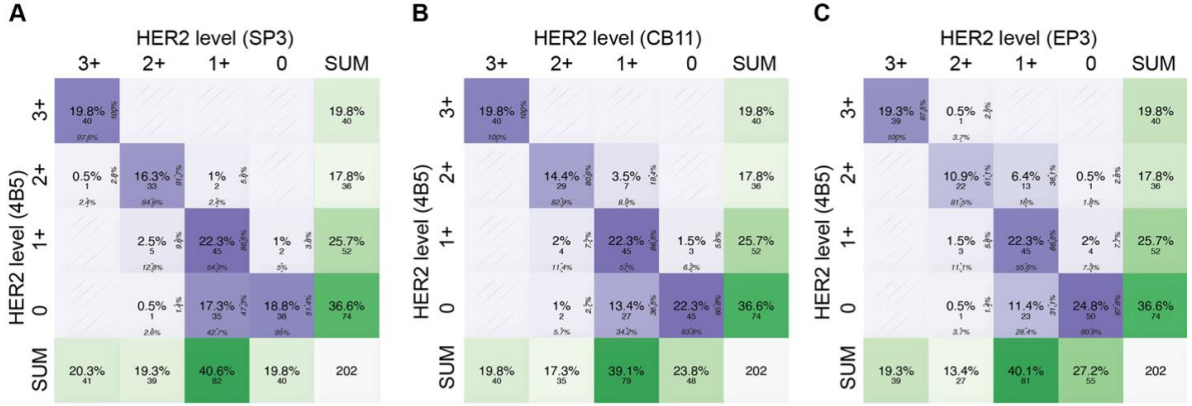
Correlation between the identified HER2 low status and therapeutic efficacy may be more important than the sensitivity

Correlation Study of Four HER2 Immunohistochemical Staining Assays in Breast Cancer and Changes of Heterogeneity Patterns [2]

Table 1. Reliability and agreement parameters for different IHC assays compared to 4B5

	percentage agreement	unweighted kappa (95%CI)	weighted kappa[1] (95%CI)	ICC[2] (95%CI)
CB11	78.72%	0.71(0.64,0.79)	0.90(0.86,0.93)	0.90(0.86,0.93)
SP3	77.23%	0.70(0.62,0.77)	0.90(0.87,0.93)	0.90(0.83,0.93)
EP3	77.23%	0.69(0.61,0.77)	0.89(0.86,0.93)	0.89(0.86,0.92)

ICC: inter-class correlation coefficient; CI: confidence interval
[1] quadratic weighted kappa
[2] two-way random effects model



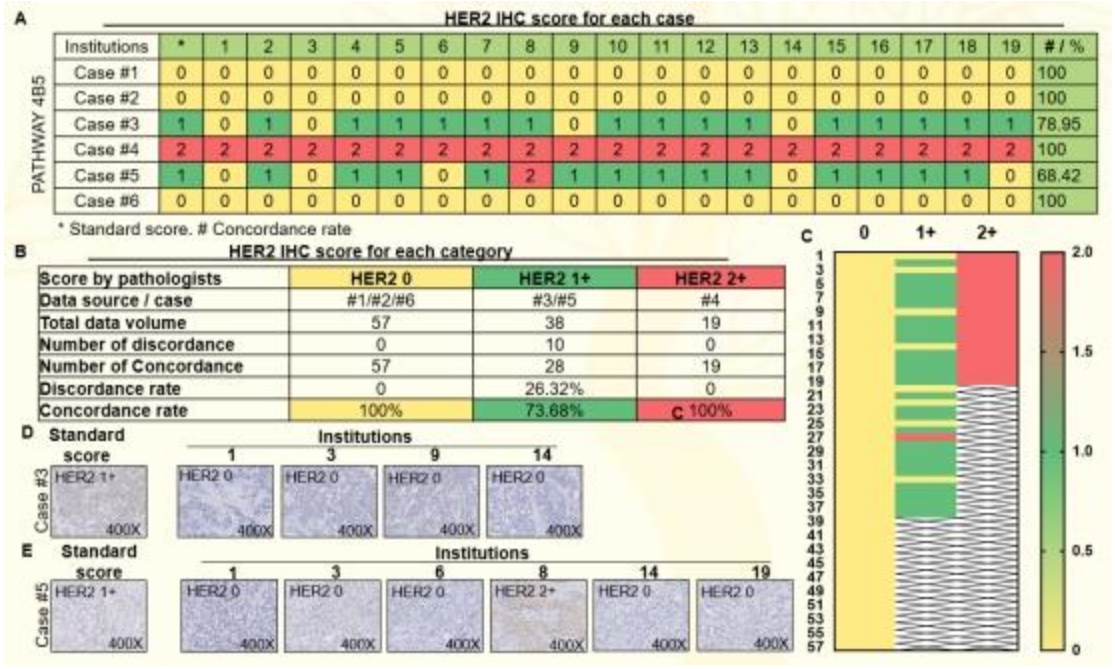
1. Rüschoff, Josef, et al. "*Virchows Archiv* (2022): 1-10.
2. Meng Yue, Yueping Liu, et al. 2024 USCAP Breast Pathology Abstract 264

Analytical：Different Staining Parameters of Same Antibody and Platform can Lead to different HER2 Results?

272 **Impact of Staining Parameters on Final Results in HER2-Low Breast Cancers-a Multi-Institutional Study of 19 Labs Using Pathway 4B5 Assay**

Ping Zhu[1], Hong Lv[2], Ming Li[2], Yizhi Zhao[3], Chenglu Zhu[4], Mingzhen Lin[5], HanSheng Li[6], Lirui Yang[2], Wenhua Jiang[7], Xu Cai[2], Wentao Yang[2]

[1]Fudan University Shanghai Cancer Center, Fudan University, Shanghai, China, [2]Fudan University Shanghai Cancer Center, Shanghai, China, [3]Hangzhou, China, [4]Westlake University, Hangzhou, China, [5]HangZhou DiYingJia Technology Co.,Ltd., Hangzhou, China, [6]Xian, China, [7]Fudan University Shanghai Cancer Center, Shanghai Medical College, Fudan University, Shanghai, China



A

HER2 IHC score for each case

PATHWAY 4B5	Institutions	*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	# / %
	Case #1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	Case #2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	Case #3	1	0	1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	78.95
	Case #4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	100
	Case #5	1	0	1	0	1	1	0	1	2	1	1	1	1	1	0	1	1	1	1	0	68.42
	Case #6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100

* Standard score. # Concordance rate

B

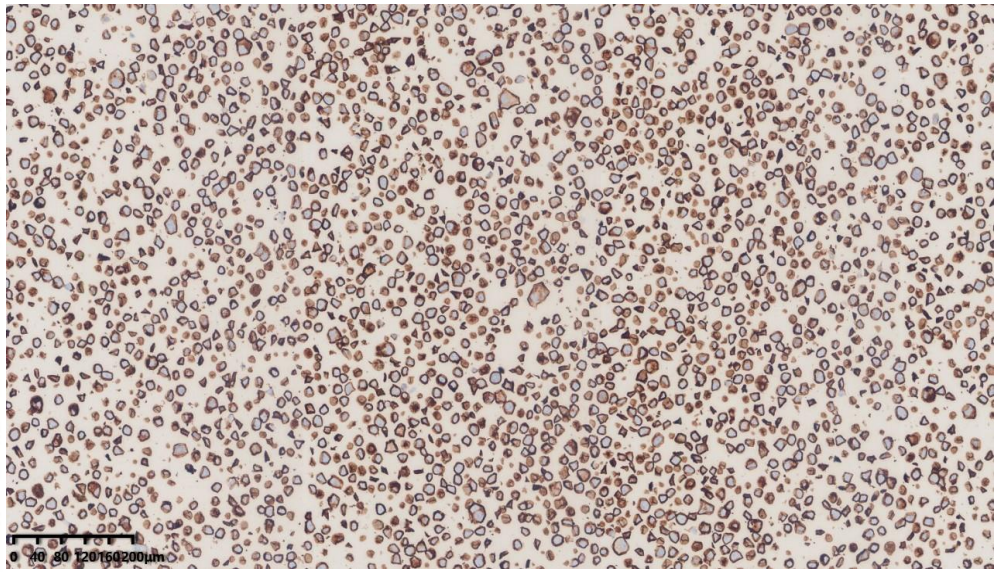
HER2 IHC score for each category

Score by pathologists	HER2 0	HER2 1+	HER2 2+
Data source / case	#1/#2/#6	#3/#5	#4
Total data volume	57	38	19
Number of discordance	0	10	0
Number of Concordance	57	28	19
Discordance rate	0	26.32%	0
Concordance rate	100%	73.68%	C 100%

- 19 institutions performed IHC staining on the same set of six cases. All laboratories employed the PATHWAY 4B5 anti-HER2 antibody and the BenchMark platform.
- Parameters such as dewaxing temperature, antigen retrieval time, and primary antibody incubation time varied among institutions.
- There were certain differences in the percentage of cell membrane staining and staining intensity for each case.
- **For antibody like 4B5, which is used as a companion diagnosis, differences in staining parameters can also affect the final result**

Ping Zhu, Wentao Yang, et al. 2024 USCAP Breast Pathology Abstract 272


U104 HER2(4B5)
1
PUNCH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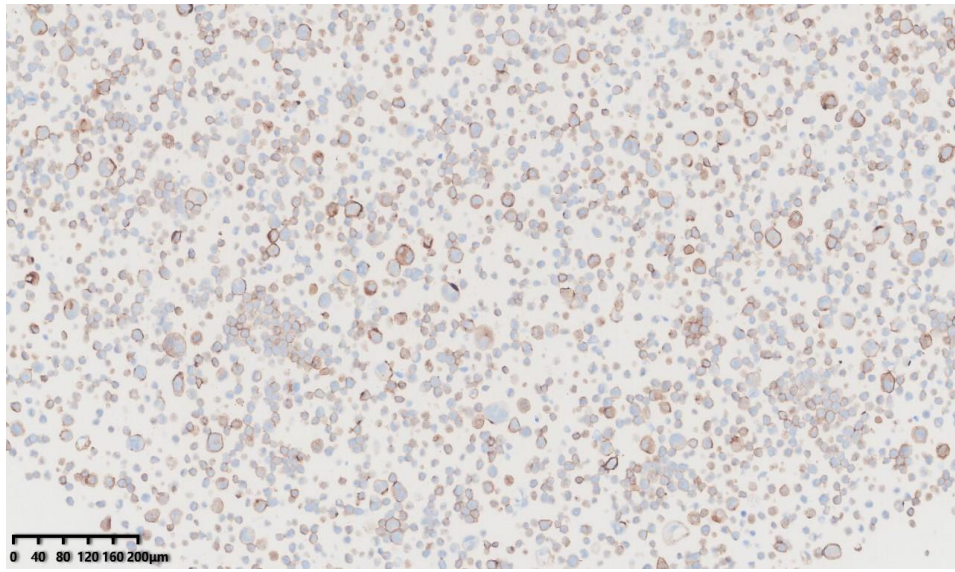
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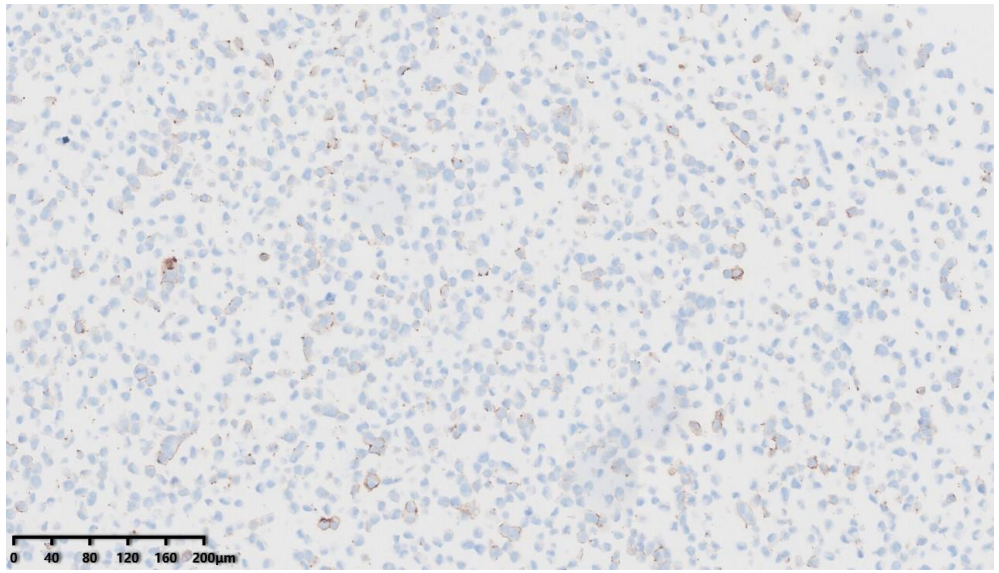
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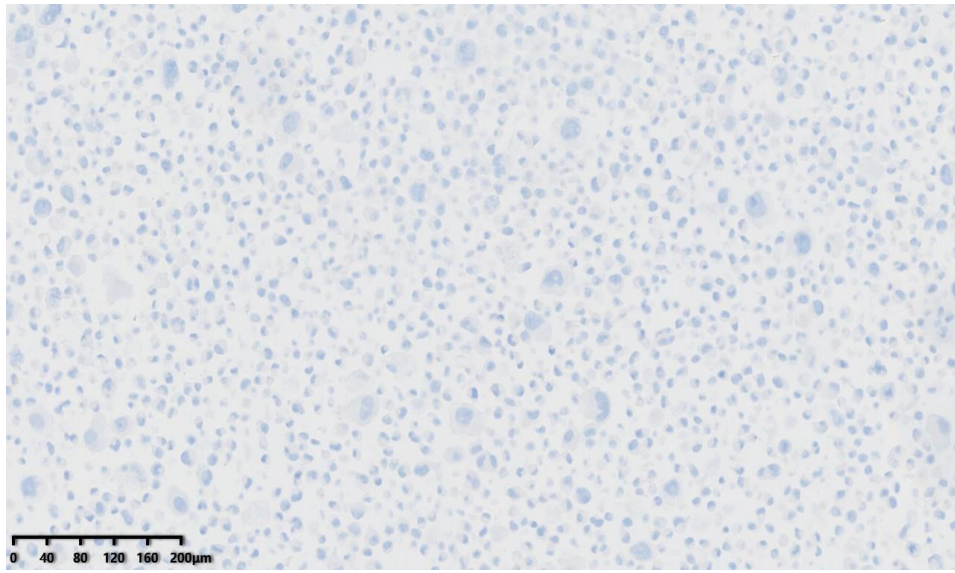
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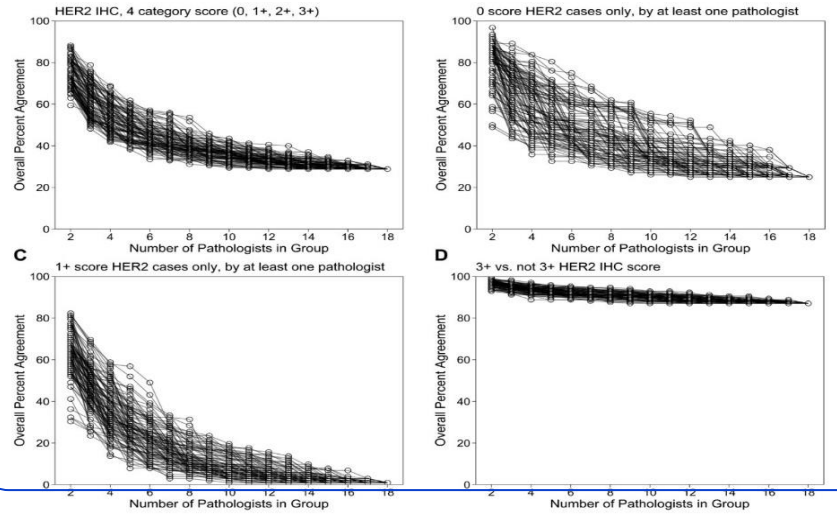
Interpretation： Challenges in the Interpretation of HER2 Low/ultra-low for Pathologists

Concordance of HER2-low scoring in breast carcinoma among expert pathologists

Levels of agreement across standard & clustered categories.

Levels of Agreement	Individual categories				Combined categories				
	0	1+	2+	3+	0	1/2/3	0	1/2	3+
Absolute	3; 6%				22; 44%		15; 30%		
	0	0	0	3					
High	23; 46%				21; 42%		27; 54%		
	3	9	10	1					
Low	19; 38%				6; 12%		7; 14%		
	3	11	4	1					
Challenging	5; 10%				1; 2%		1; 2%		
	1	3.5	0.5						
Cases/category	7	23.5	14.5	5	7	43	7	38	5

- **16 expert pathologists** of the UK National Coordinating Committee for Breast Pathology scored 50 digitally scanned HER2 IHC slides[1].
- Highest concordance (86%) was achieved when scores were clustered as 0 versus others



- **18 pathologists** from
- 15 institutions scored HER2 IHC in 170 breast cancer biopsies [2]
- As the number of pathologists evaluating increases, the concordance of all evaluation groups decreases.

What is the consistency in the interpretation of HER2 ultra-low?

Analysis results of the American population [3]

Concordance among two pathologists' (P1/P2) review of IHC biopsy slides.

	P2 No Observable IHC Staining	P2 "HER2 Ultra-Low"	P2 IHC 1+	P2 IHC2+/ Indeterminate	P1 Total
P1 No observable IHC staining	104 κ=0.47	23	2	0	129 (43%)
P1 "HER2 Ultra-Low"	48	54 κ=0.17	2	0	104 (35%)
P1 IHC 1+	7	44	11 κ=0.21	0	62 (21%)
P1 IHC 2+/ Indeterminate	1	0	2	2 κ=0.57	5 (2%)
P2 Total	160 (53%)	121 (40%)	17 (6%)	2 (1%)	300
				Concordance	171 (57%)
				Discordance	129 (43%)

- After adding the HER2 ultra-low subgroup, the overall interpretation consistency rate of two pathologists is 57%.
- The main discordance in interpretation are between "IHC 0" and "ultralow".

Analysis results of the Chinese population [4]

Shanghai Cancer Center

	null	ultralow	1+
Null	53	9	2
Ultralow	14	13	8
1+	2	8	60

- In the HER2 Path study
- Among the samples previously evaluated as IHC 0 by 9 sub-centers and Fudan Cancer Center, the overall interpretation concordance is 74.1% (158/229).

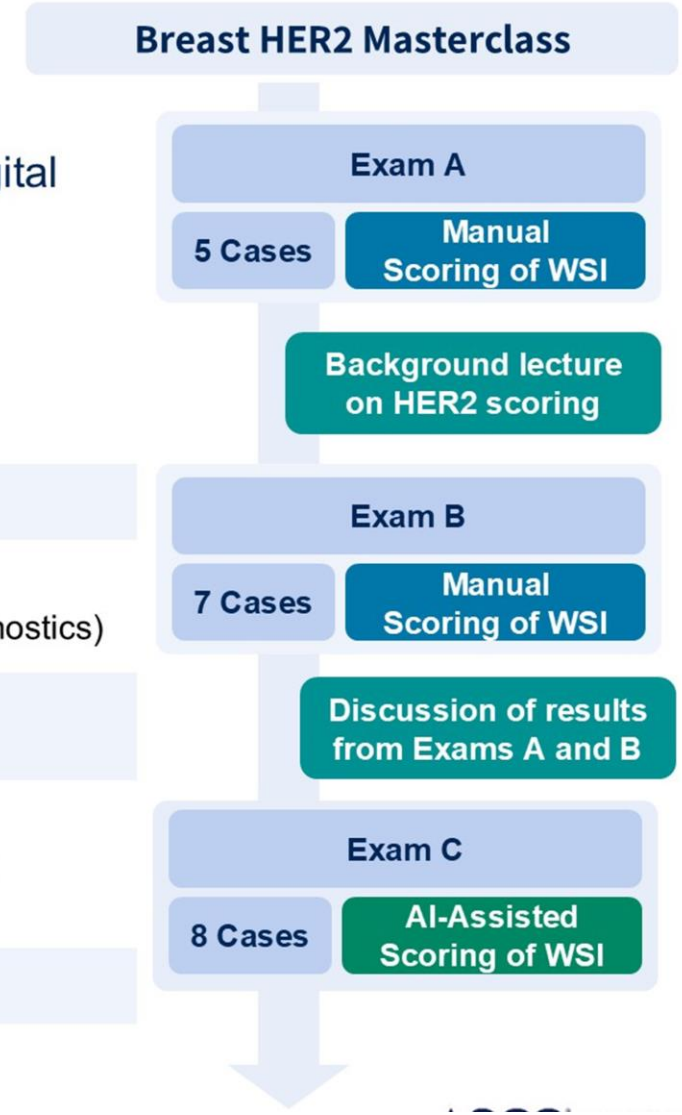
1. Zaakouk M, Quinn C, Provenzano E et al. Breast. 2023 Aug;70 82-91
2. Robbins CJ, Fernandez AI, Han G, et al. Mod Pathol. 2023 Jan;36(1):100032.
3. Sandhya Mehta, et al. J Clin Oncol 42, 2024 (suppl 16; abstr e13156)
4. Wentao Yang, et al. 2023 SABCS, PO4-26-08

Interpretation： HER2 AI could increase HER2 ultralow interpretation concordance

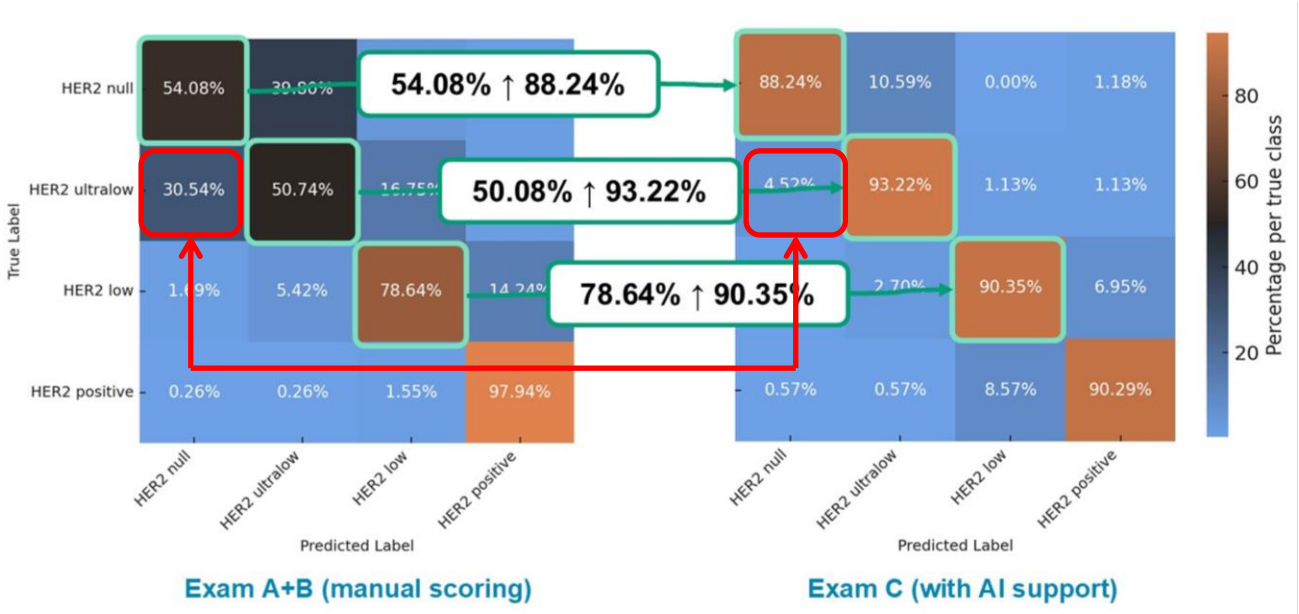
Methods

- Pathologists participated in masterclass sessions, assessing 20 digital HER2 IHC-stained breast cancer cases **w/ *vs.* w/o AI assistance**
- Cases assigned **ground-truth IHC scores** by a **central reference center** were divided into 3 exams: A, B, and C
- The AI software was used for decision support **only for Exam C**

Dataset	105 pathologists / 10 countries
Cases	20 digital HER2 IHC-stained breast cancer samples IHC: HER2 4B5 Pathway Ventana® Assay (Roche Diagnostics)
Ground Truth	Central reference center assigned IHC scores
Scoring Criteria	ASCO/CAP 2023 guidelines plus: ● HER2-Ultralow: IHC 0 with any membrane staining ● HER2-Null: IHC 0 with no membrane staining
Explainability	AI provided per-cell HER2 classifications



Interpretation： HER2 AI could increase HER2 ultralow interpretation concordance



- AI support **raises sensitivity** across HER2 Null/Ultralow/Low expression classifications
- HER2-Ultralow underscoring manually in 30.5% of instances, **compared to 4.5%with AI**

➢ **Conclusions:**

- AI-assisted training improved pathologists' concordance in HER2 IHC score and clinical categories
- AI reduced the misclassification of HER2-Low and HER2-Ultralow cases as HER2-Null by24.4%, potentially enabling more patients to access HER2-directing ADC therapies
- These findings highlight the value of AI systems in biomarker interpretation training, providing pathologists with enhanced decision-making tools at the individual cell level and improving diagnostic precision in HER2 IHC interpretation

Wolff AC, et al. *Arch Pathol Lab Med.* 2023;147(9):993-100

Regarding HER2 low/ultralow, What are the interpretation rules and pitfalls?

“Magnification Rule”: Differentiation HER2 0 with or without membrane staining under X40 objective

4X

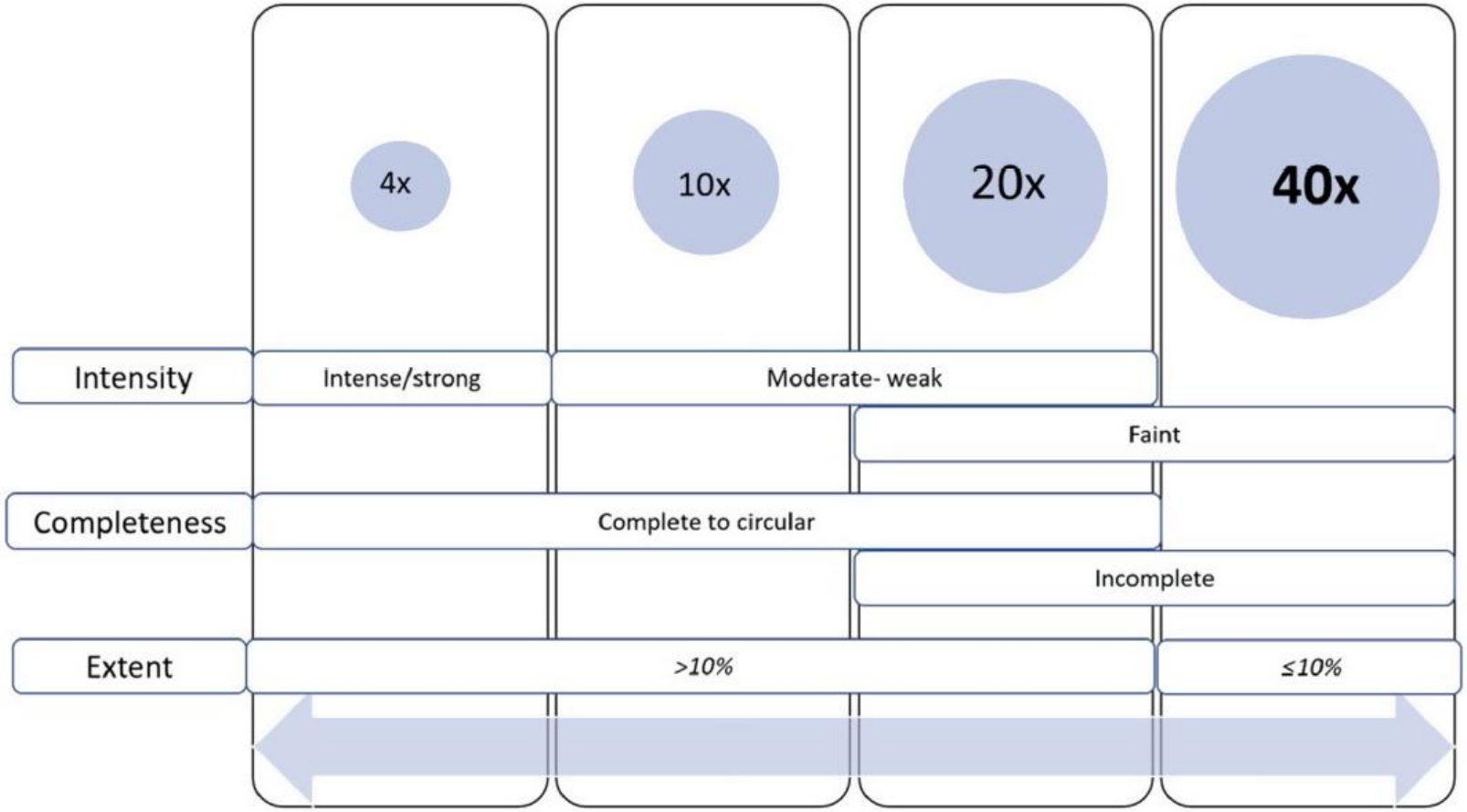
10X

20X

40X

Intensity	Magnification
Strong staining	Strong membrane staining that is clear under low magnification (×4 to ×5)
Moderate intensity	Membrane staining clearly visible under ×10 magnification
Weak intensity	Membrane staining clearly visible under ×20 magnification
Faint barely perceptible staining	Faint and barely visible membrane staining can only be observed at the high magnification (×40)

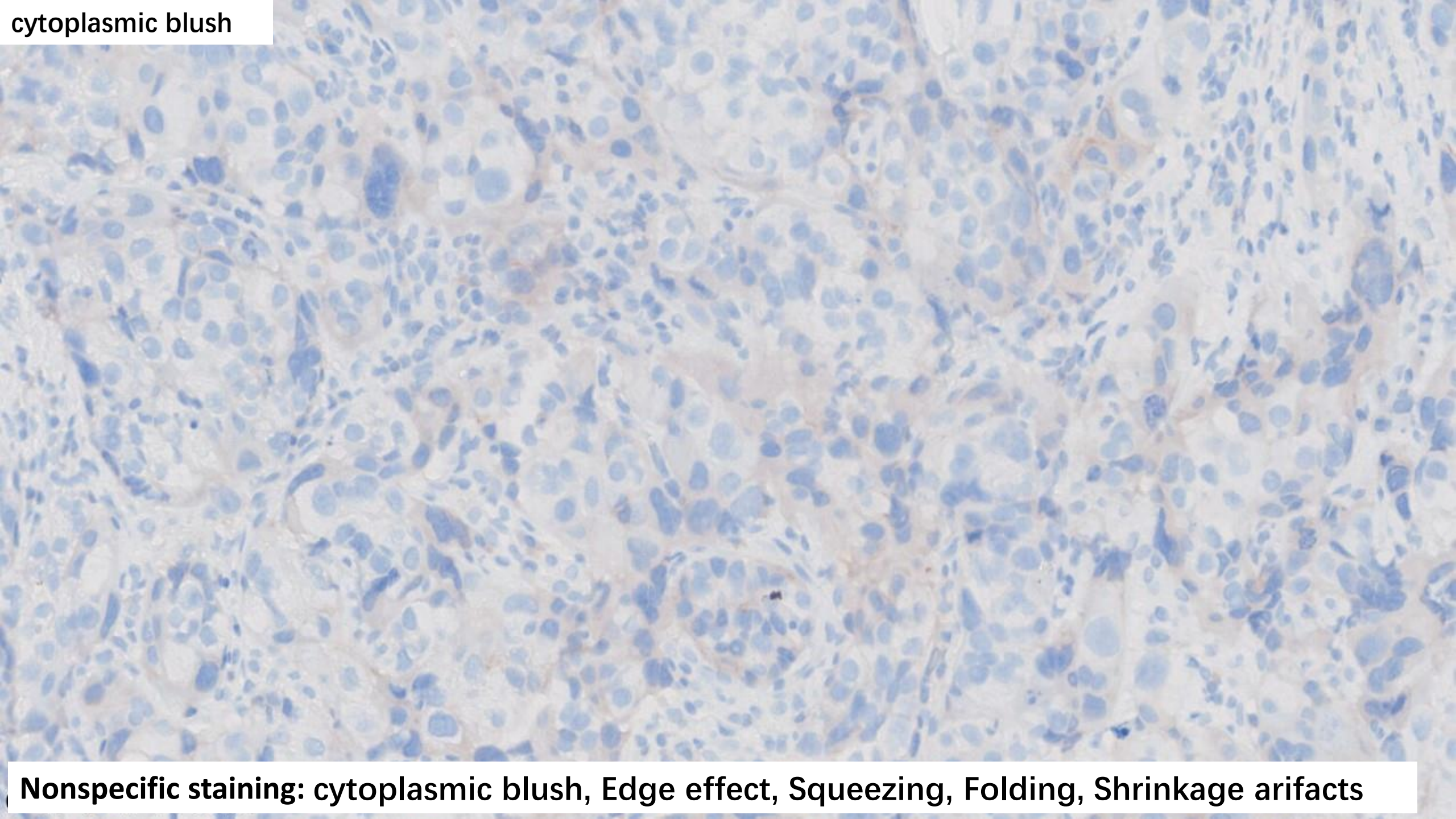
The ‘I-C-E (intensity–completeness–extent of staining)’ framework can be consulted as a practical approach for HER2 IHC scoring.



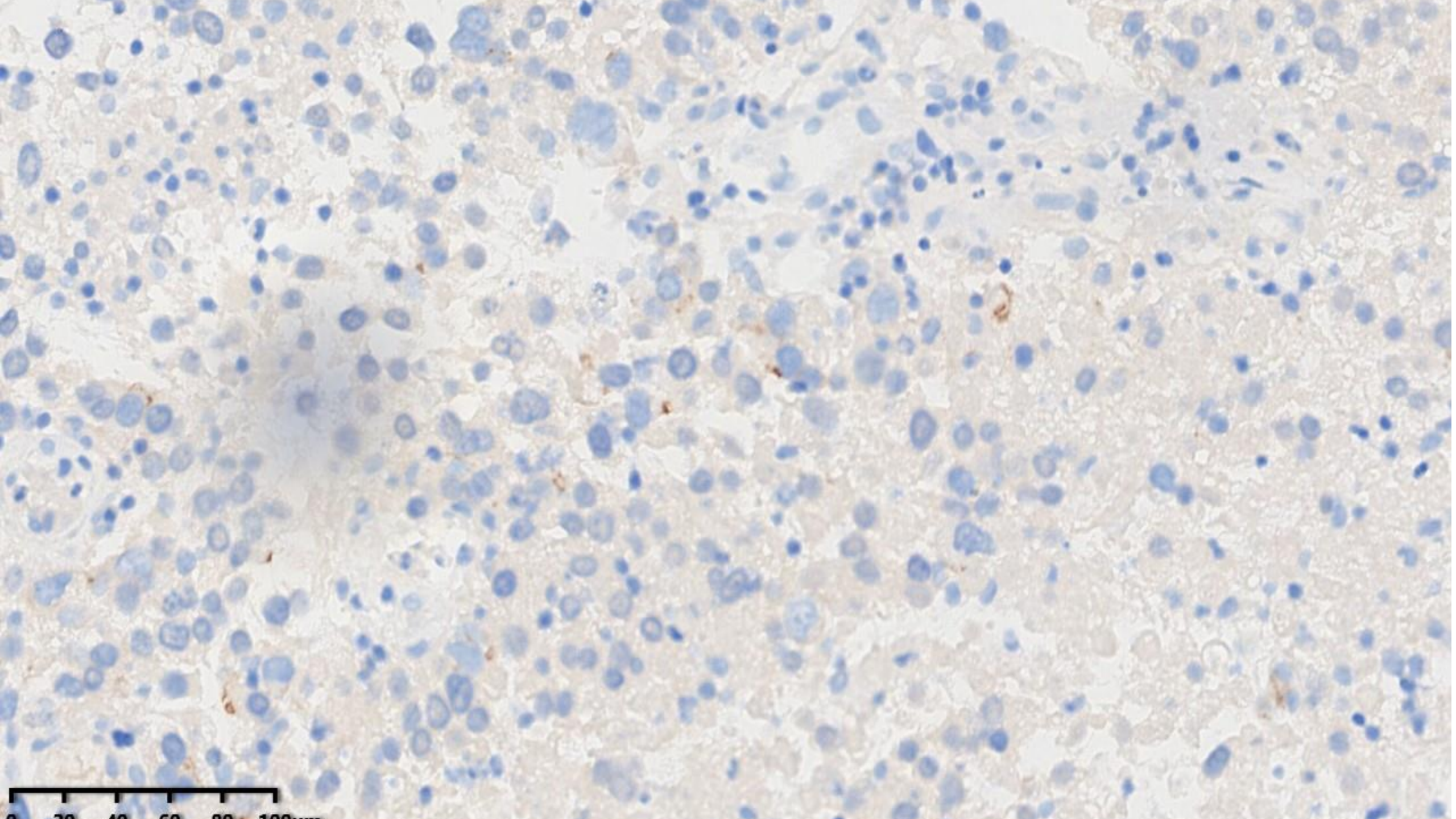
Proposed decision aid that can be used to evaluate HER2 testing at the lower spectrum.

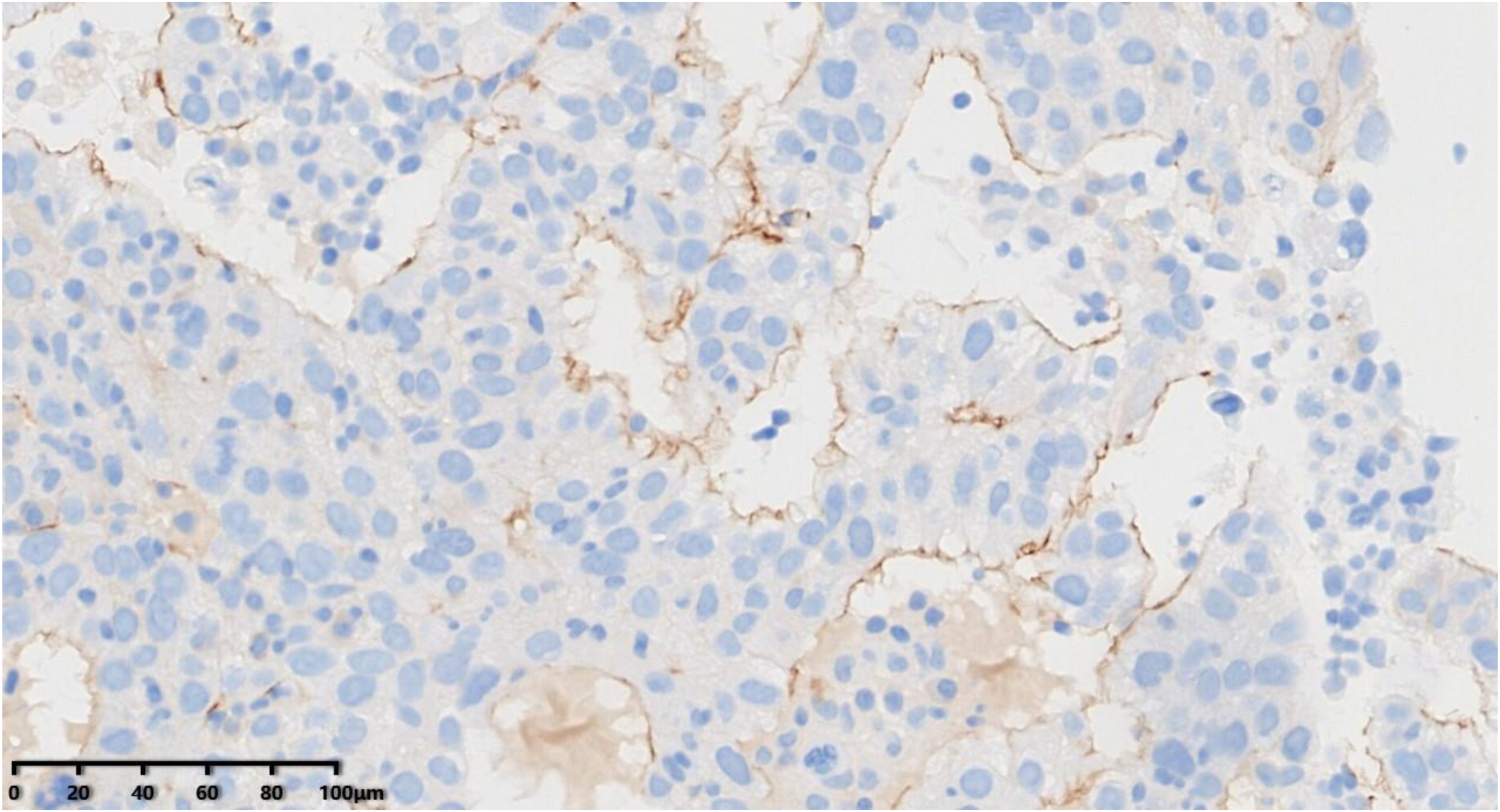
- **I: Intensity** of staining
- **C: Completeness** of staining
- **E: Extent** or proportion scoring is required for categorization.

Tan PH, Mihir G, Laokulrath N, Rakha E. Practical approach to scoring HER2 immunohistochemistry in breast cancer in the wake of updated guidelines. Histopathology. 2024;84(4):715-718. doi:10.1111/his.15117



Nonspecific staining: cytoplasmic blush, Edge effect, Squeezing, Folding, Shrinkage arifacts

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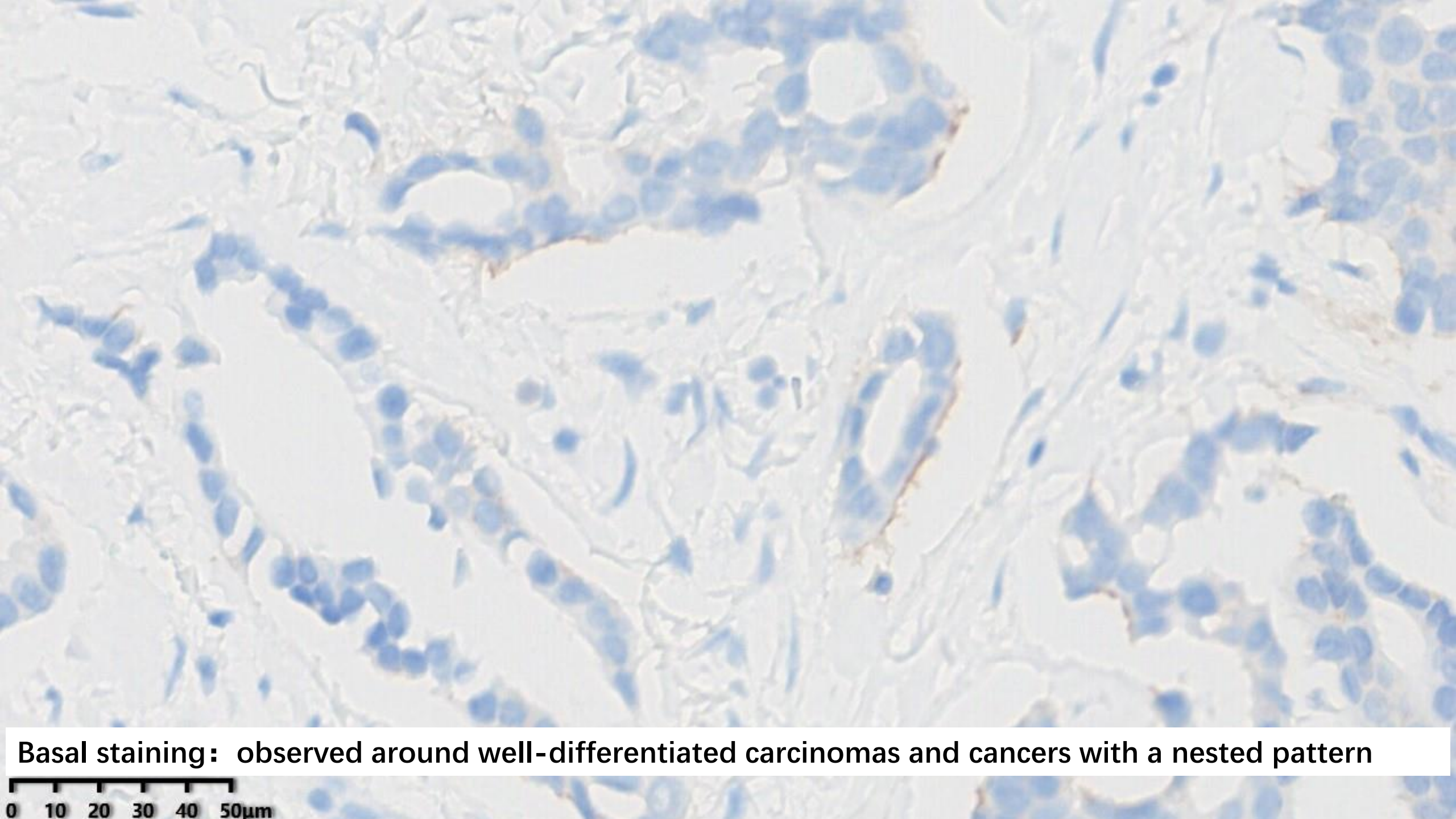
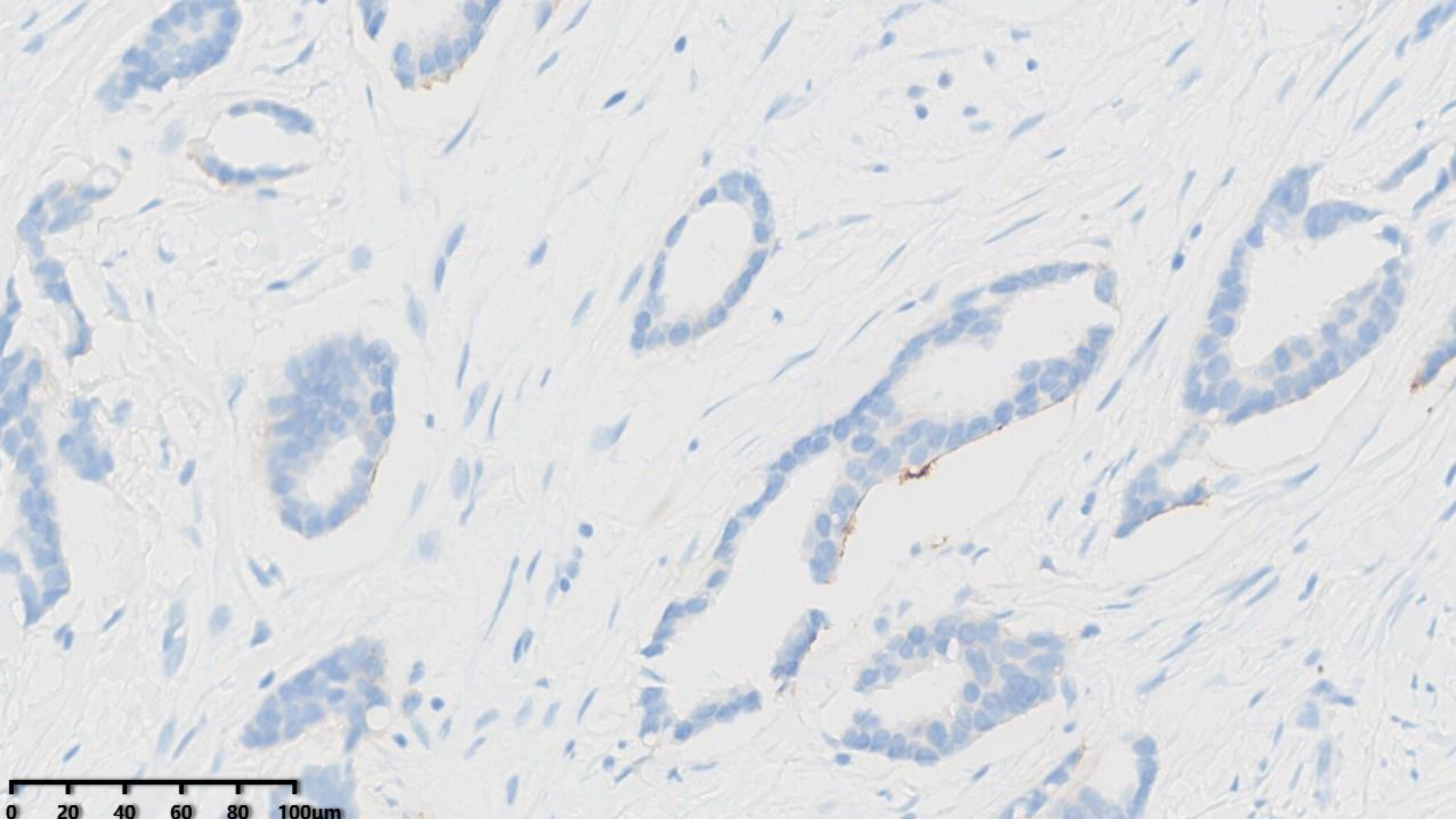


Basal staining：observed around well-differentiated carcinomas and cancers with a nested pattern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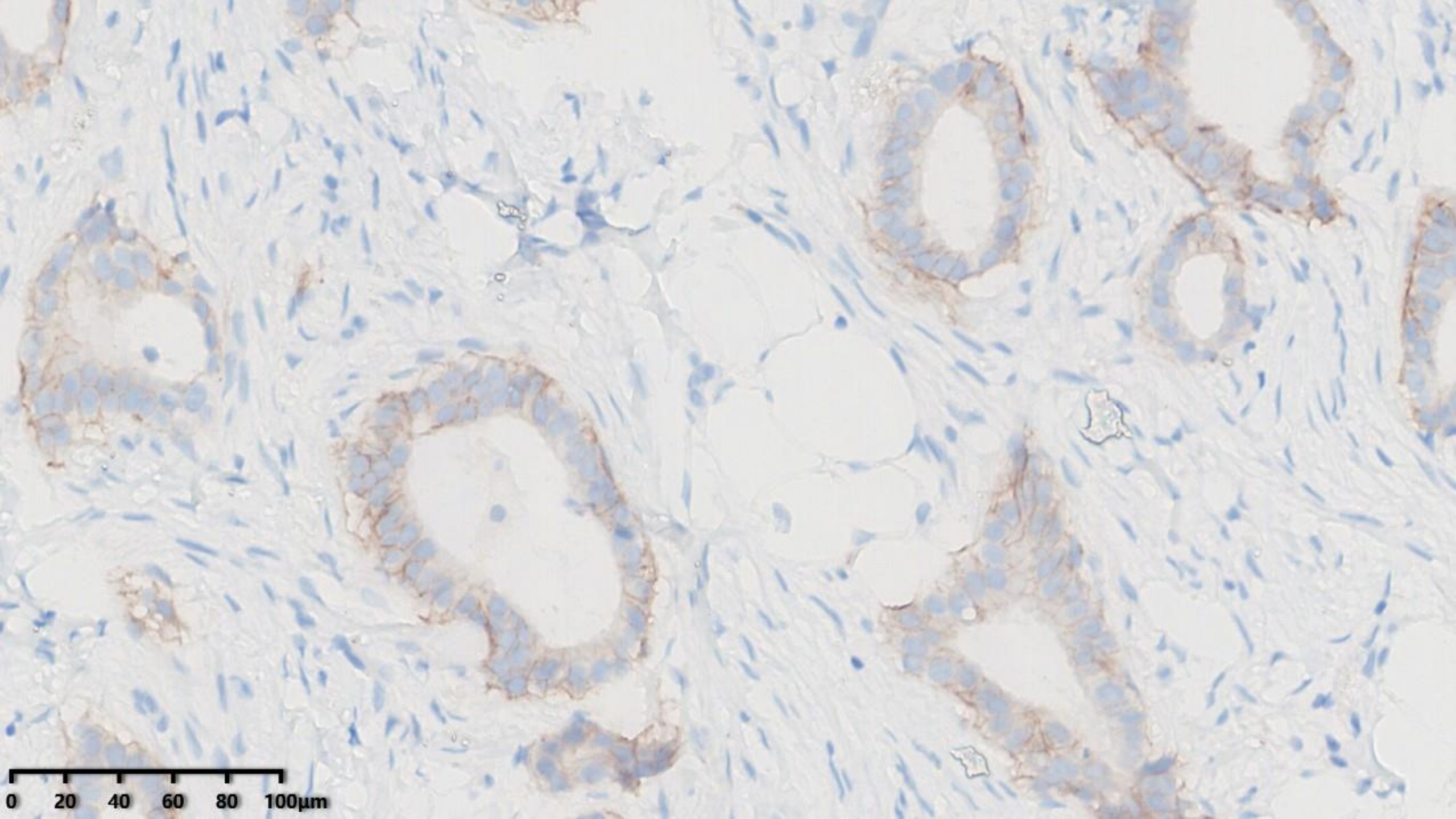
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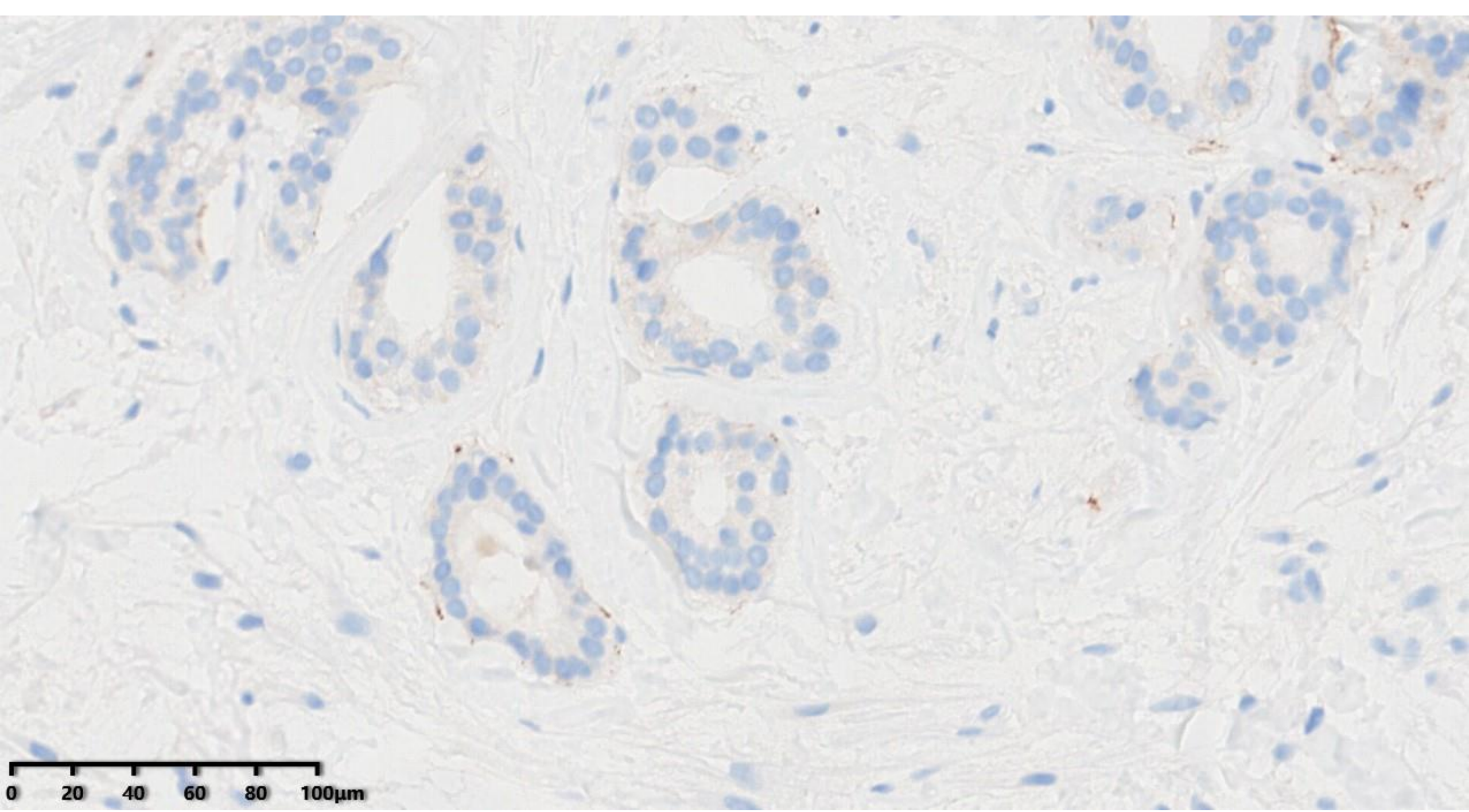
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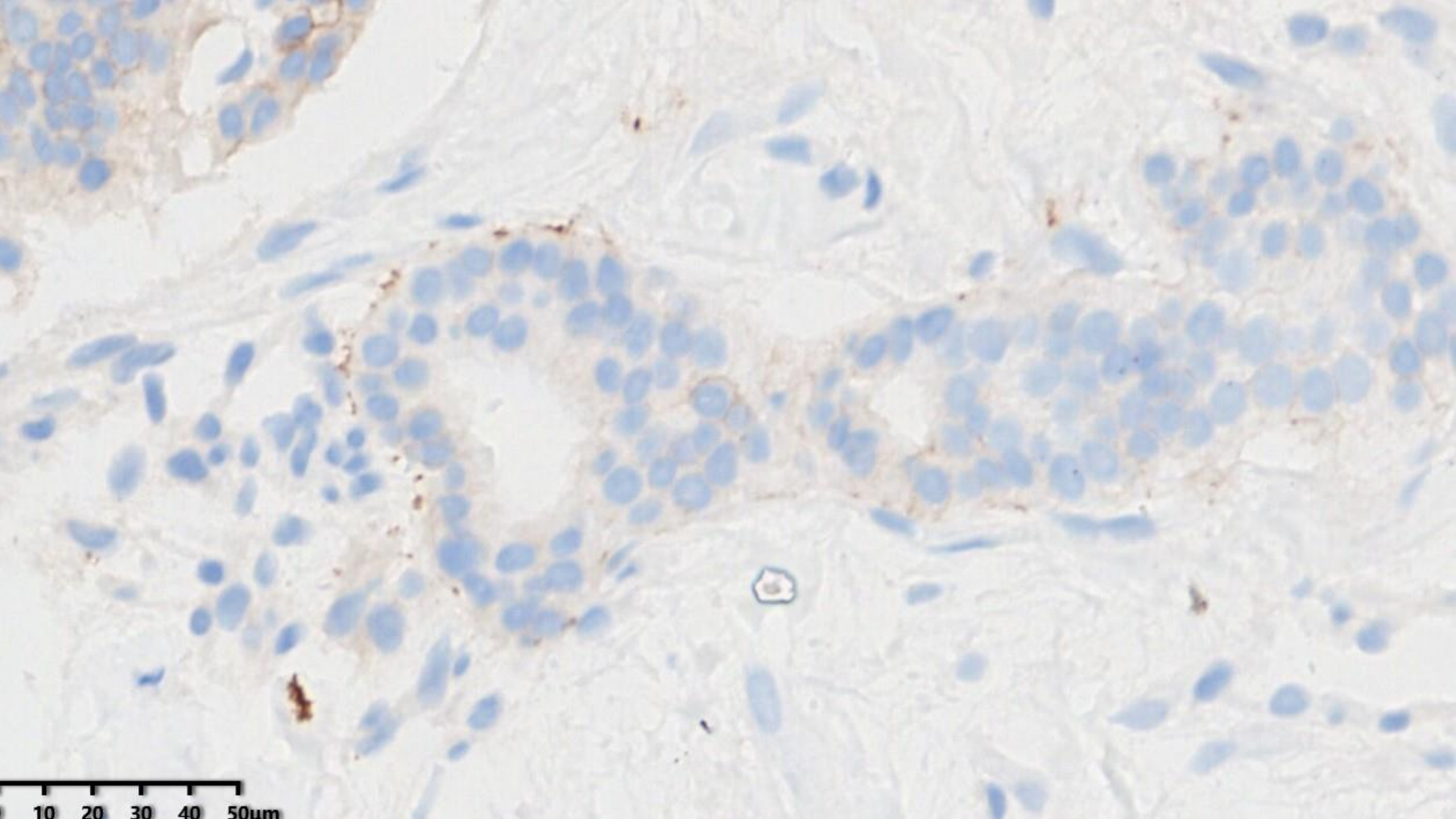
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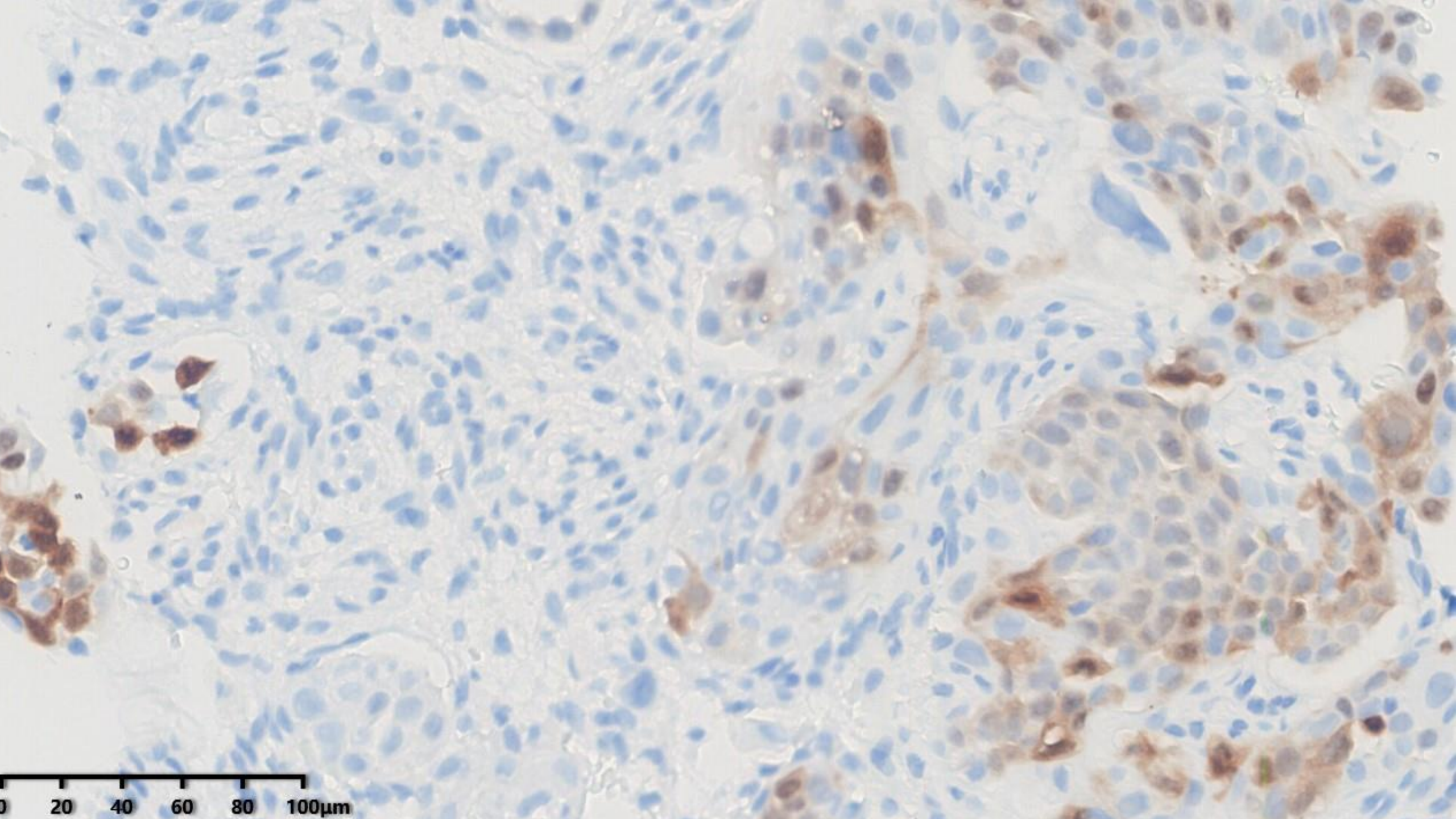
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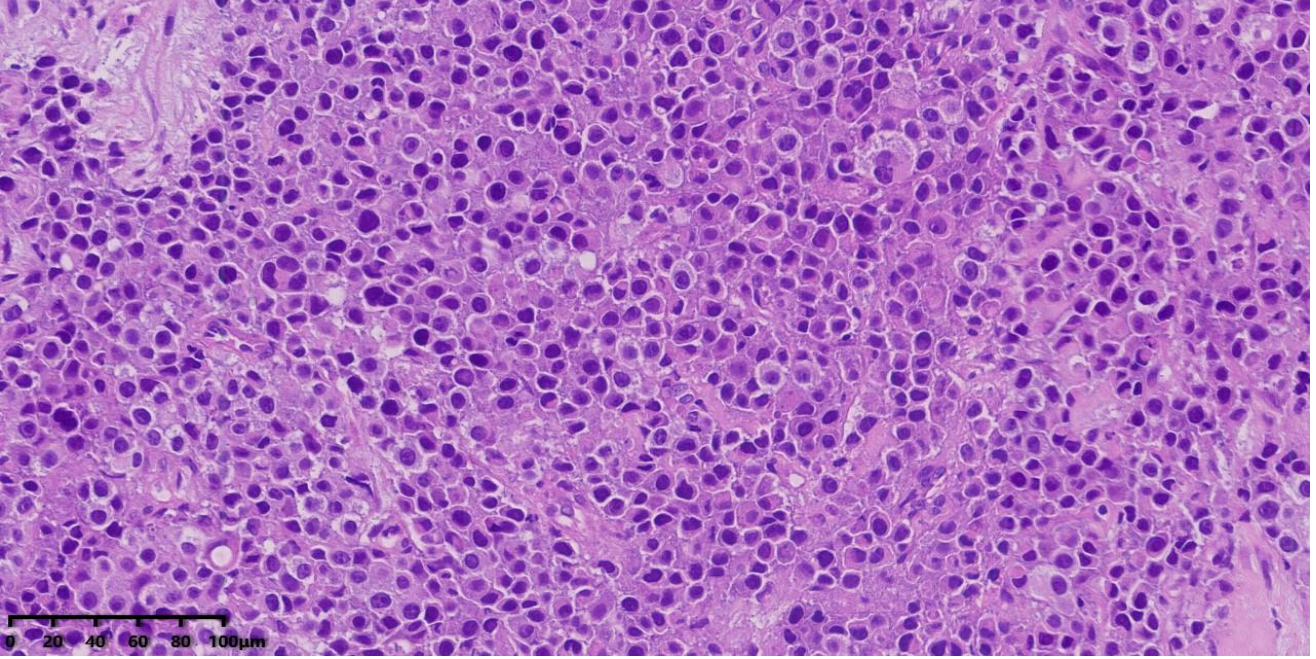
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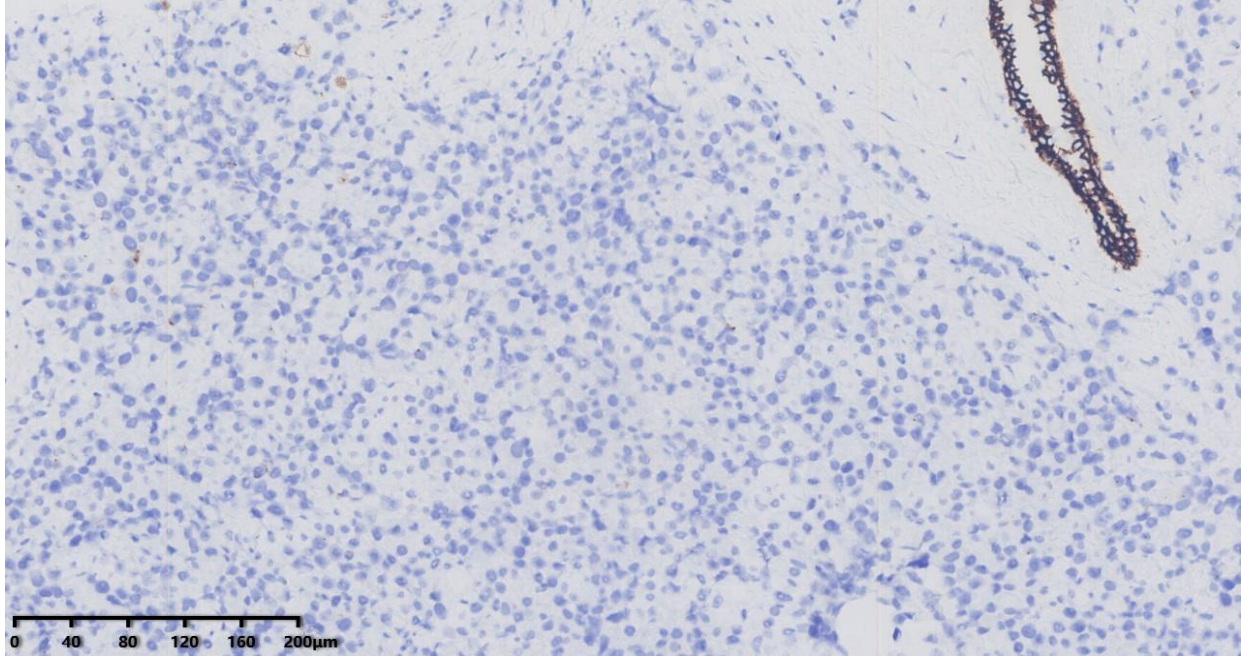
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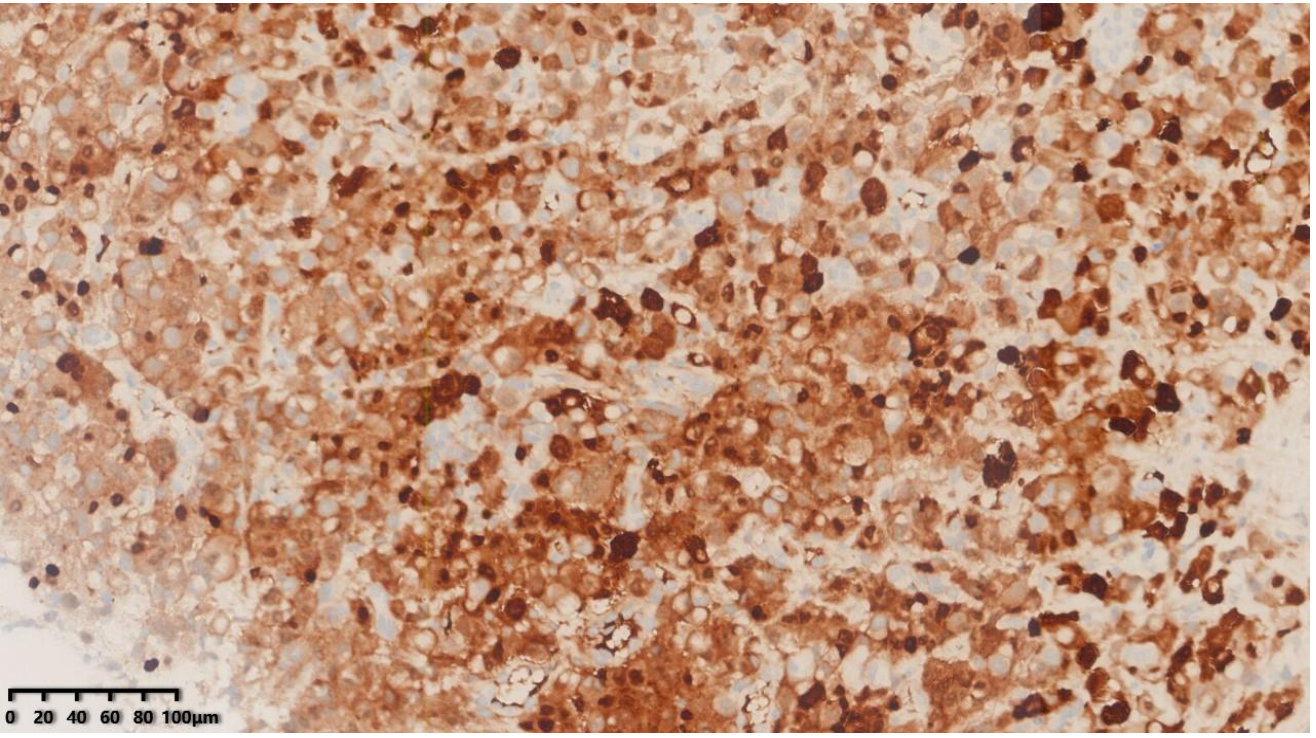
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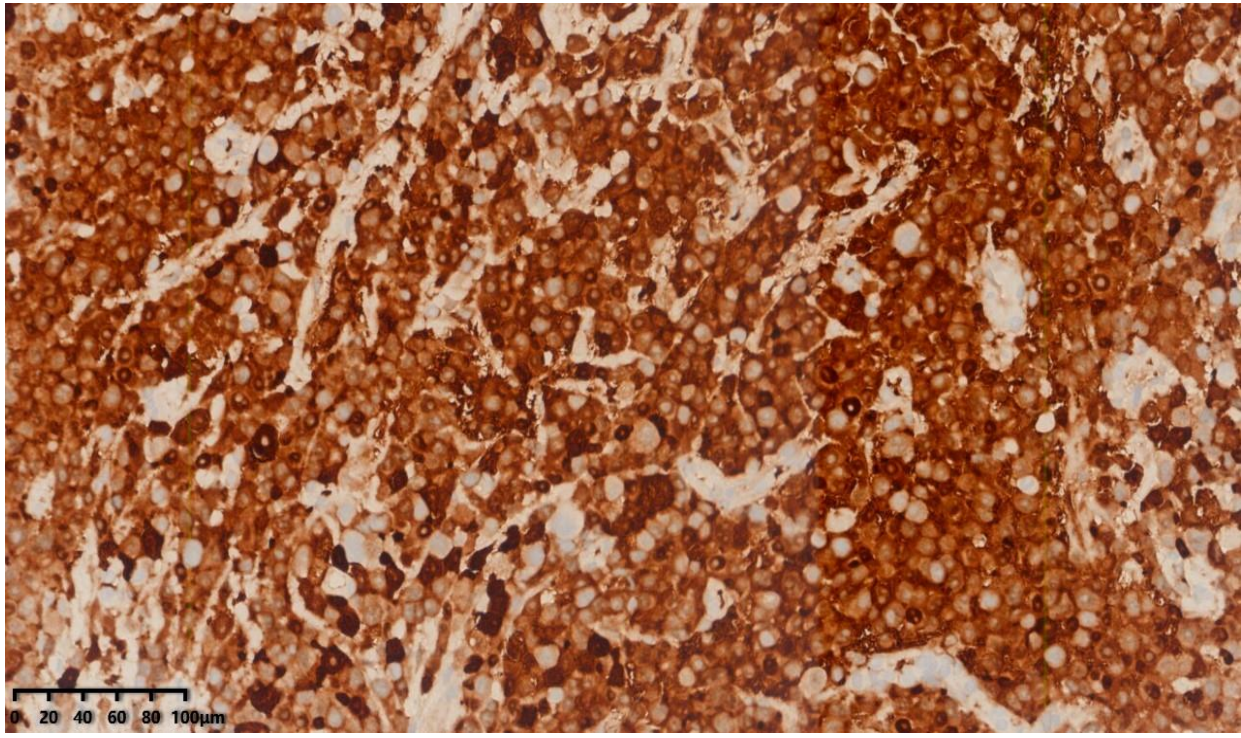
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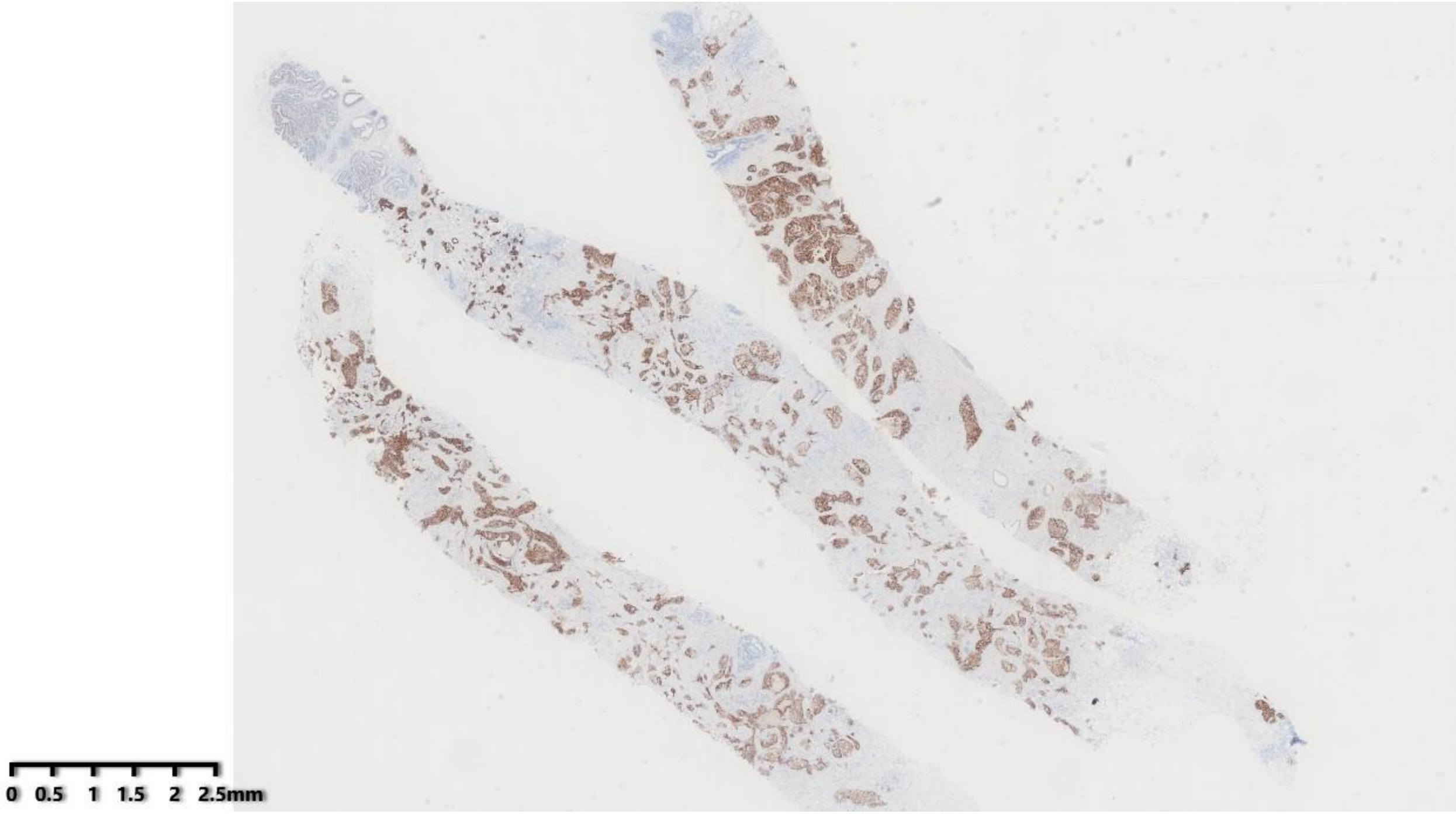
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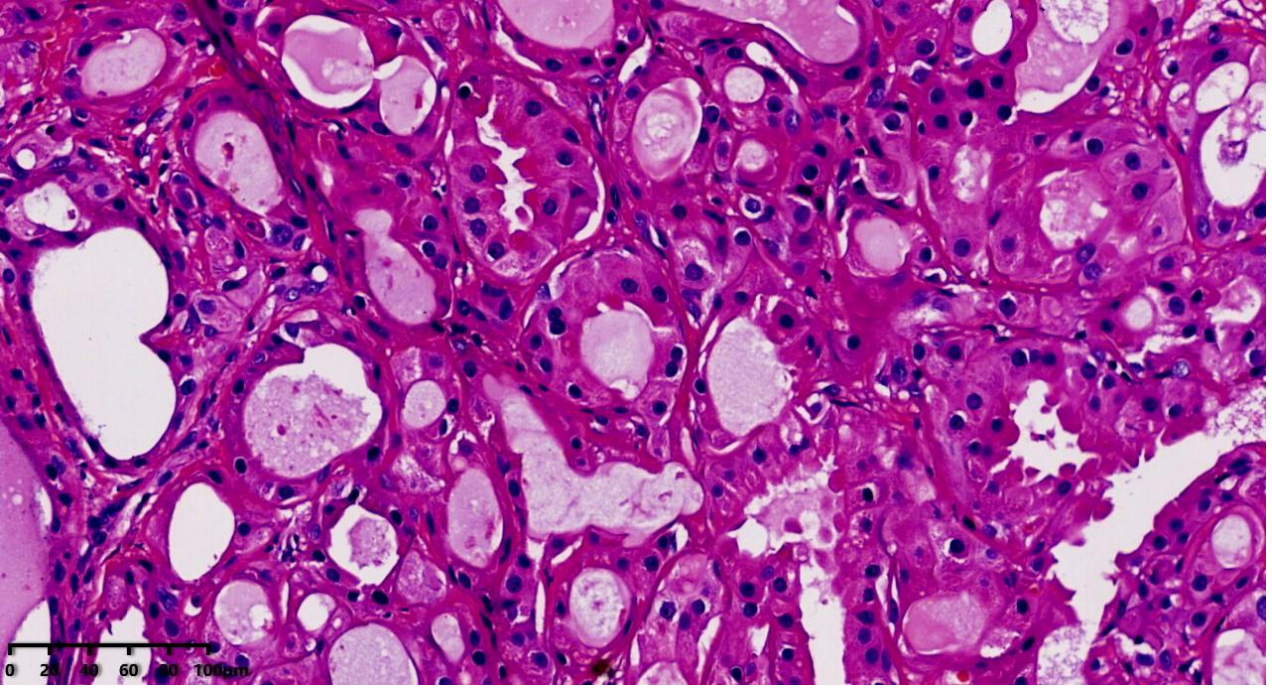
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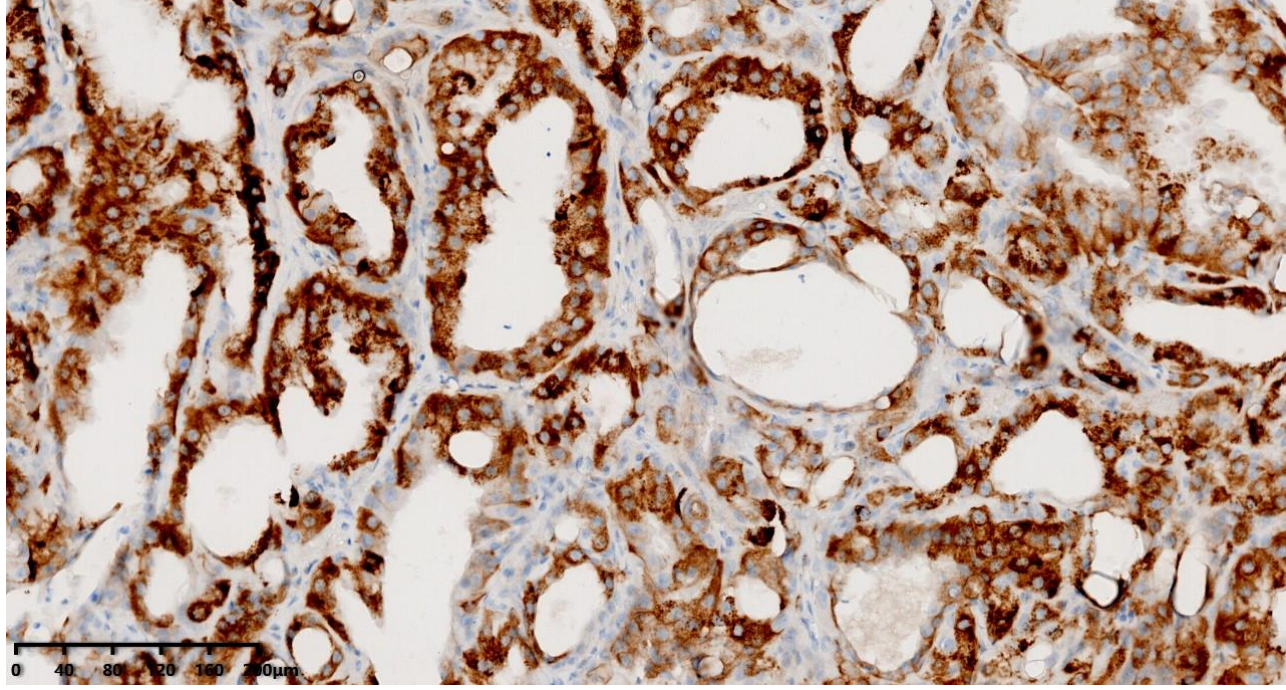
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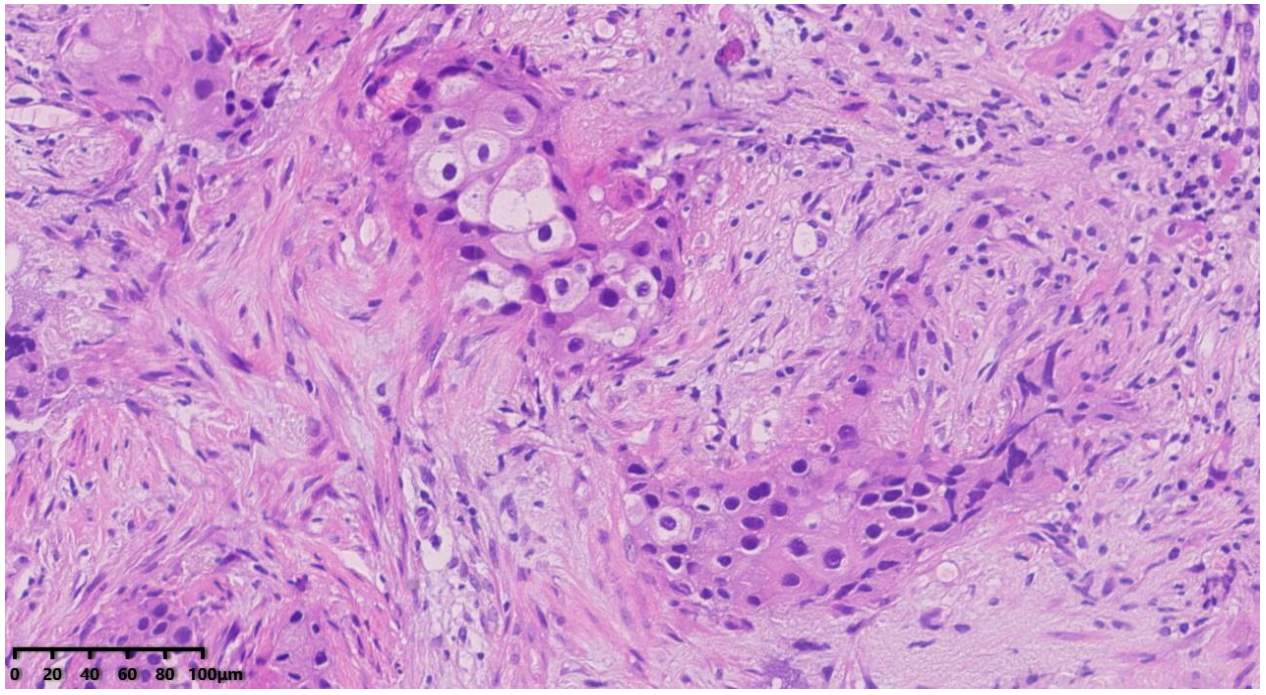
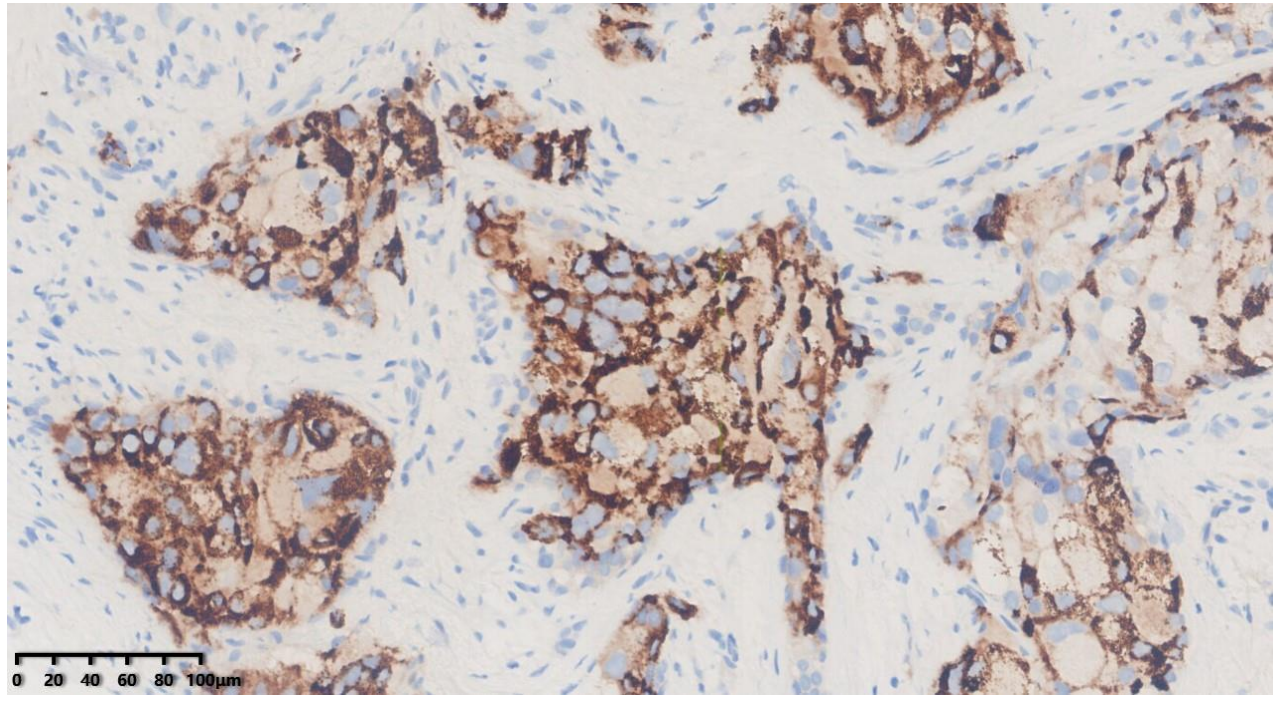
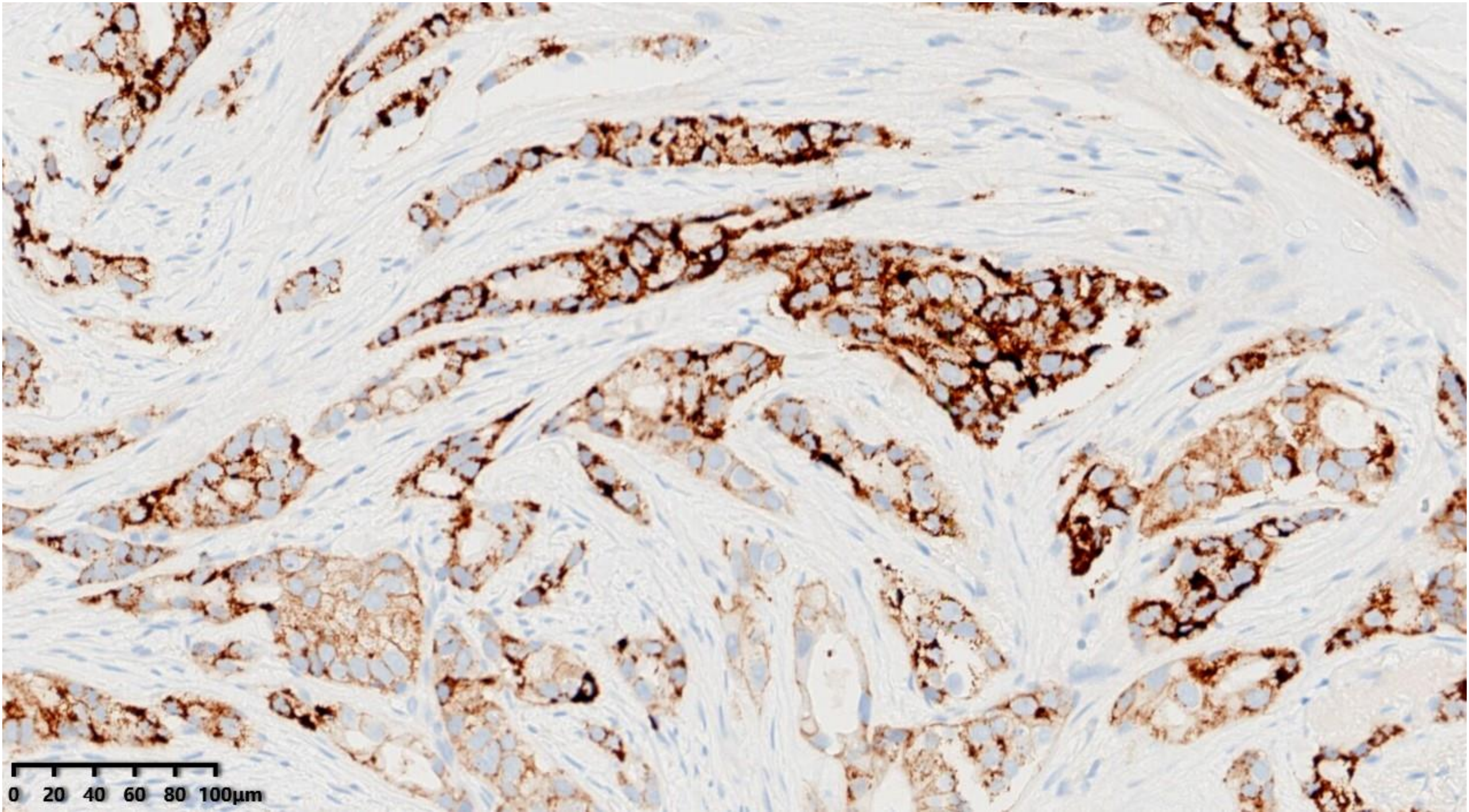
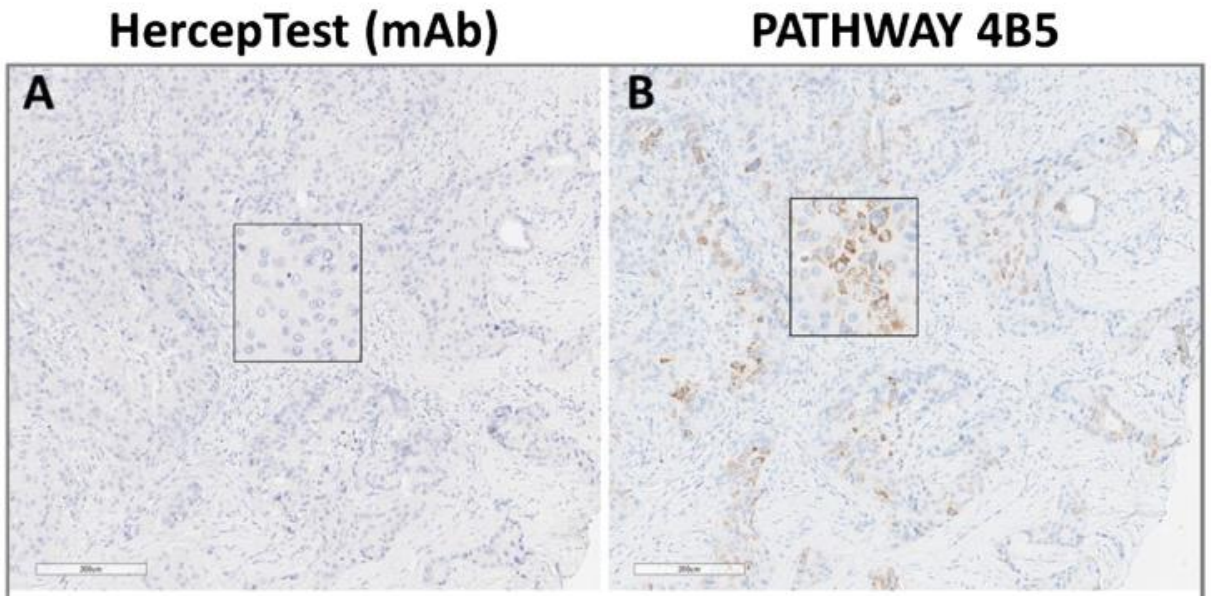

0 20 40 60 80 100µm


0 20 40 60 80 100µm


0 20 40 60 80 100µm

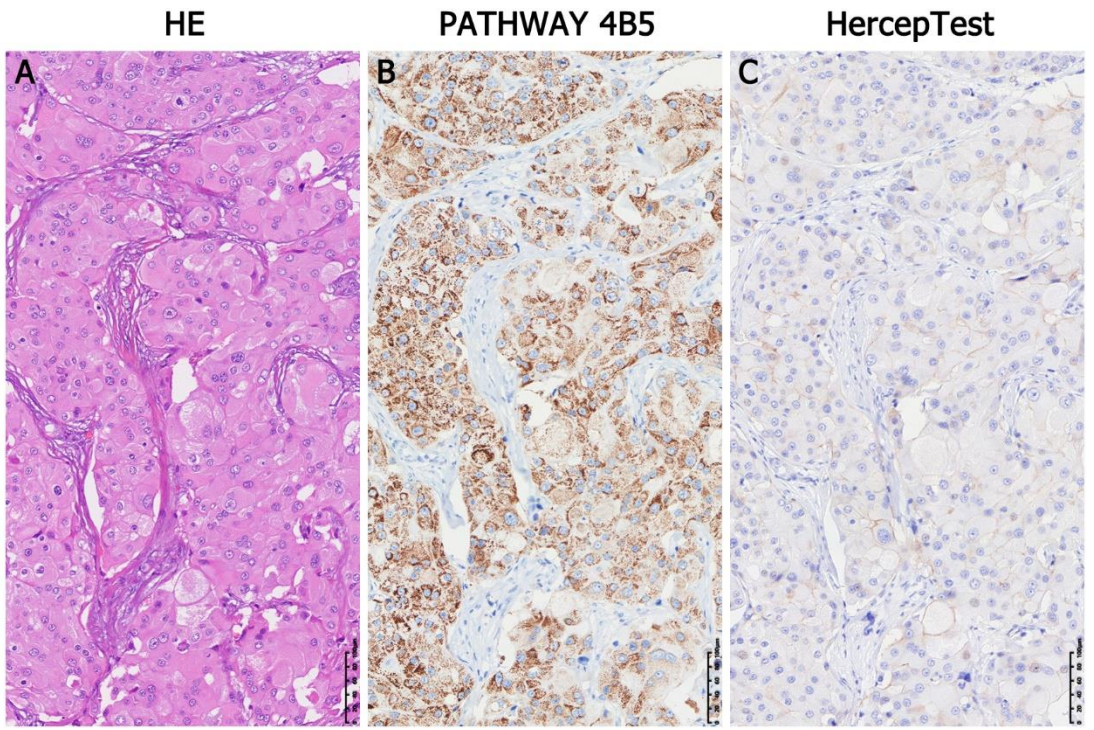
Analytical： Different Staining Platform can Lead to Different Staining Pattern



PATHWAY 4B5 staining was characterized by the occasional presence of diffuse and/or dot-like cytoplasmic staining in tumor cells

251 **Study on Different Staining Platforms for HER2 Cytoplasmic Granular Staining Pattern in Pure Apocrine Carcinoma of the Breast**

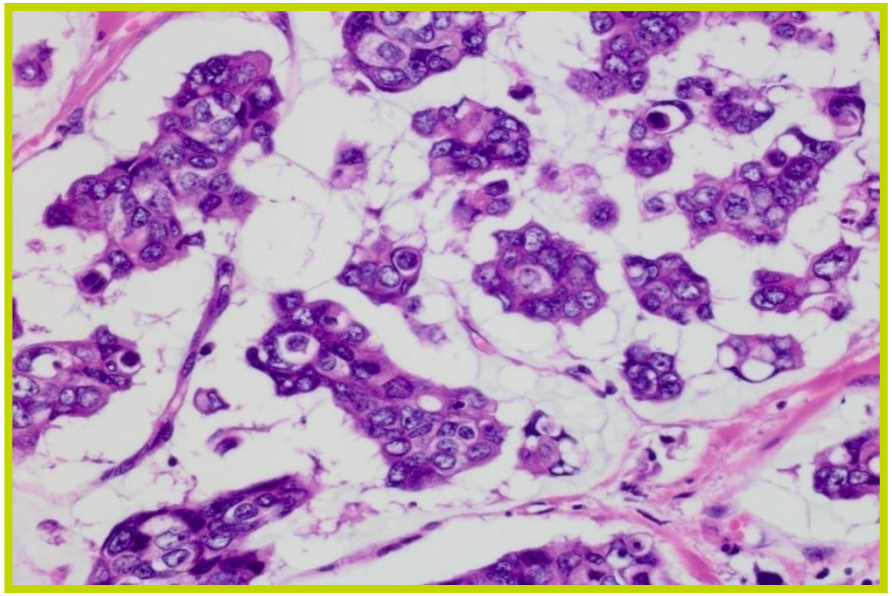
Xuexue Xiao[1], Mingwei Wang[1], Na Fang[1], Junqiu Yue[1]
[1]*Hubei Cancer Hospital, Wuhan, China*

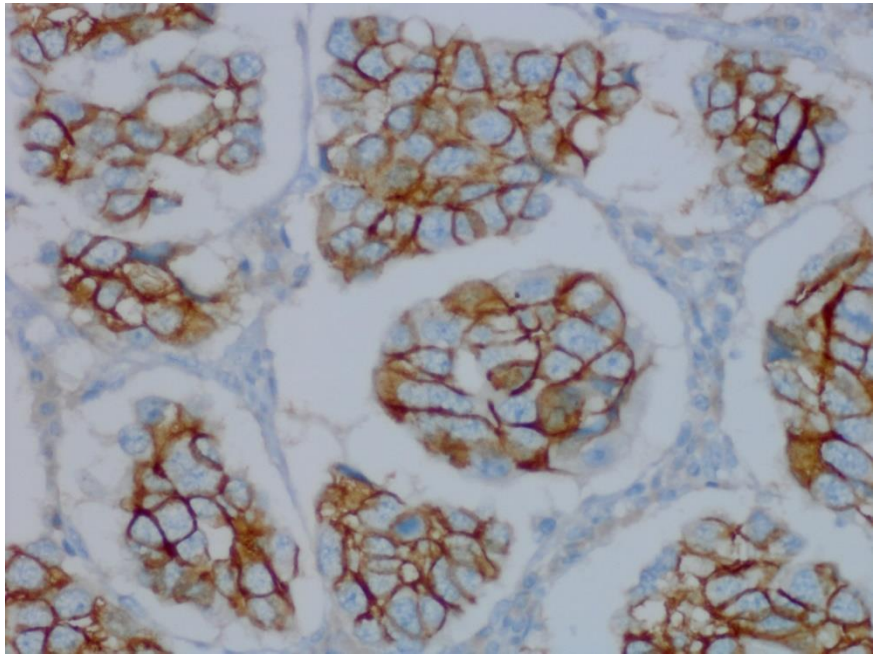


- The proportion of apocrine carcinomas of the breast with HER2 cytoplasmic granular staining is lower with HercepTest than that of the PATHWAY 4B5 platform.

Virchows Arch. 2022 Nov;481(5):685-694

basal-lateral staining





- Loss of membranous staining at the stromal interface.
- Suggests disrupted cell polarity, characteristic of invasive micropapillary carcinoma.

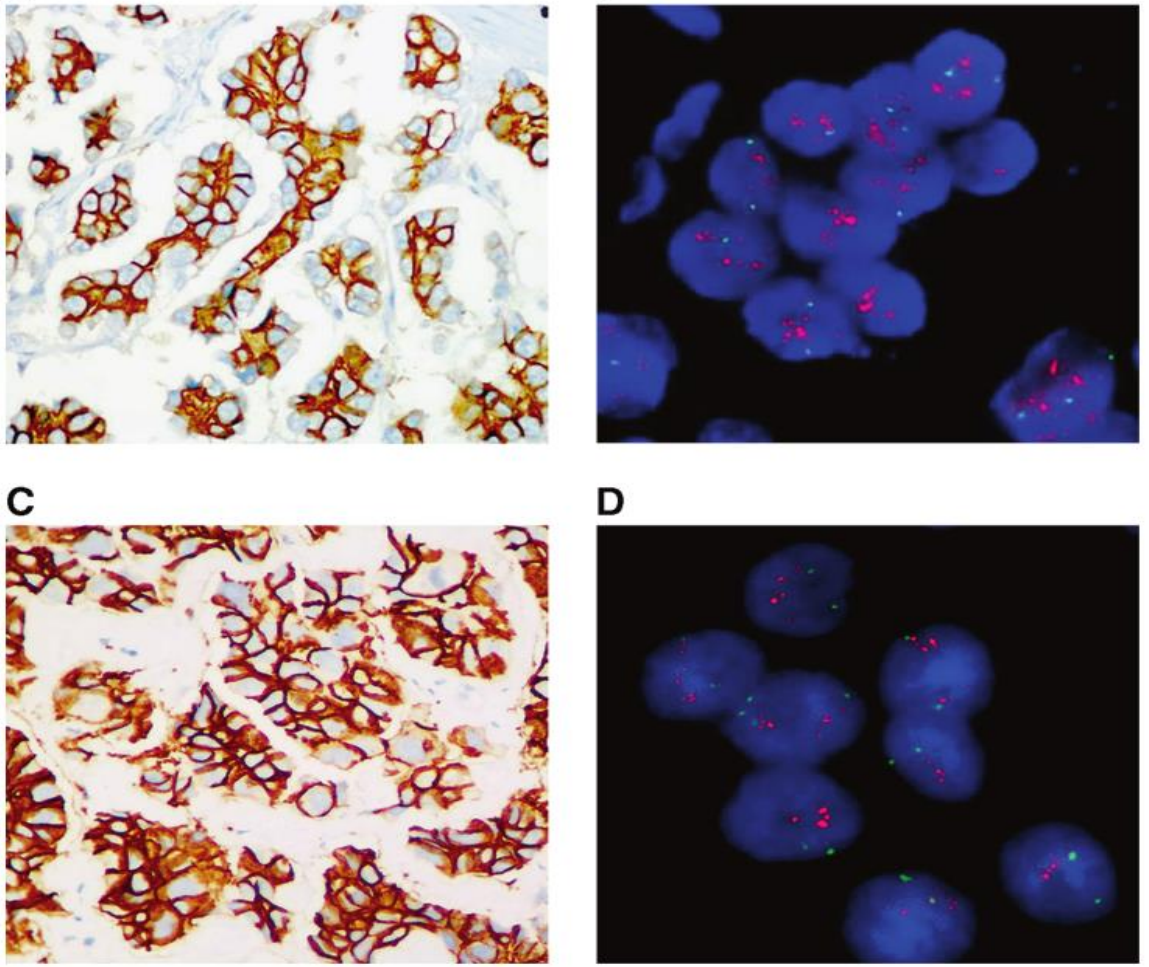
Modern Pathology
https://doi.org/10.1038/s41379-020-0461-z

USCAP

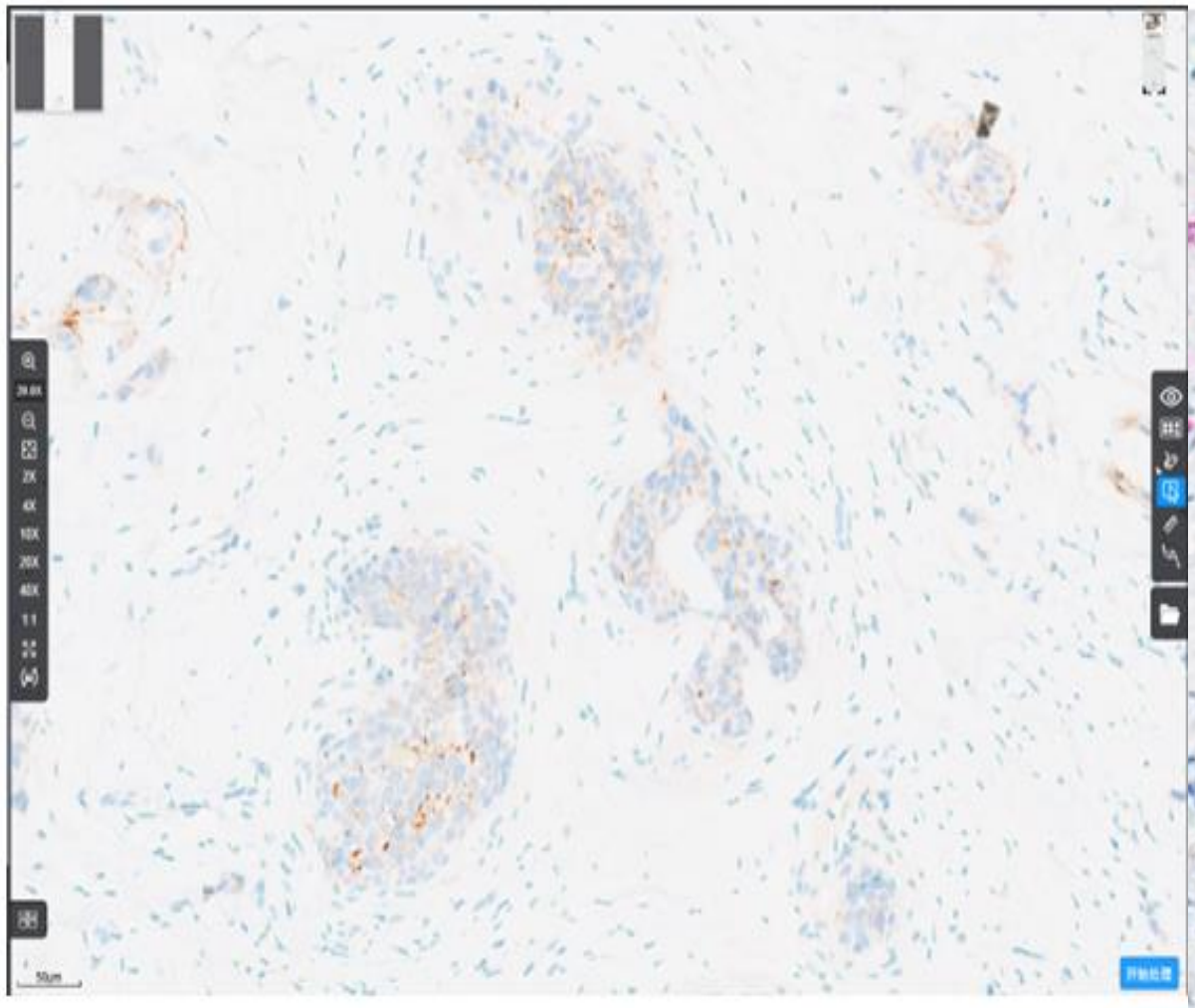
ARTICLE

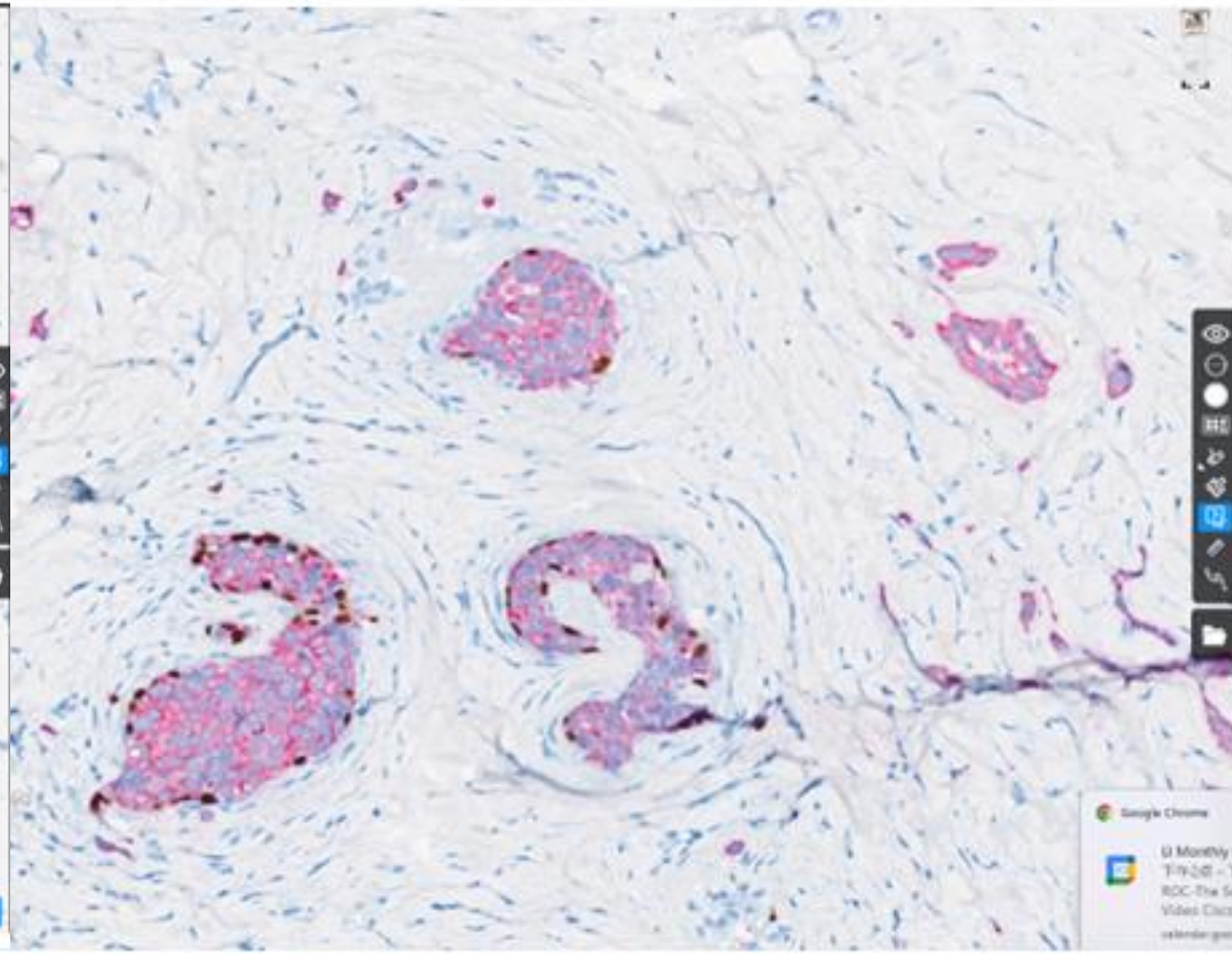
Intense basolateral membrane staining indicates *HER2* positivity in invasive micropapillary breast carcinoma

Shuling Zhou[1,2] · Fei Yang[1,2] · Qianming Bai[1,2] · Anqi Li[1,2] · Ming Li[1,2] · Siyuan Zhong[1,2] · Hong Lv[1,2] · Ruohong Shui[1,2] · Xiaoyu Tu[1,2] · Rui Bi[1,2] · Xiaoli Xu[1,2] · Yufan Cheng[1,2] · Baohua Yu[1,2] · Shaoxian Tang[1,2] · Xiangjie Sun[1,2] · Xiaoyan Zhou[1,2] · Wentao Yang[1,2]



Irregular DCIS mistaken for invasive carcinoma





Application of artificial intelligence algorithms in breast pathology

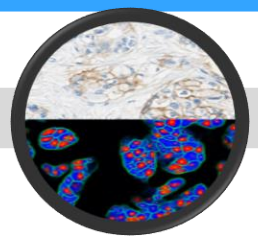
Quantitative continuous scoring (QCS) helps with Quantitative Analysis of IHC Images

Identify tumor cells through image deep learning, measure the optical density value (OD value) of tumor cell staining, re-evaluate the HER2 expression status with reference to this data, and explore and verify the threshold through clinical research.

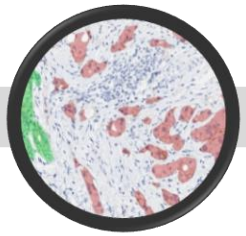
Supervised Deep Learning Image Analysis

Developed Independent of Efficiacy

Whole slide digitized image



Automated region segmentation of epithelium



Automated subcellular segmentation of tumor cell(SSTC)

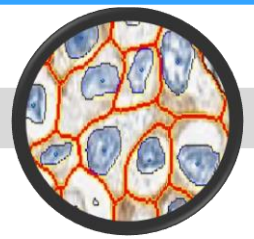


Image input

Digital images from multiple scanners can be accepted.

Image analysis data

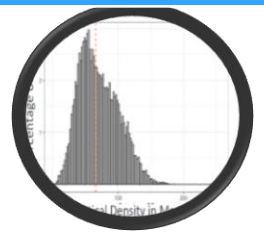
Automatically identify invasive tumors

- Automatic identification of cell membranes
- Single cell membrane OD and distance

Bioinformatics

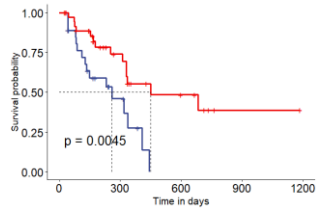
Driven by Efficacy

Analysis



QCS Data

Clinical outcome



Clinical test and validation

- Determine the optimal patient selection cutoff value
- Maximize ORR/PFS/OS

Outcomes with T-DXd treatment	HER2+ (IHC 3+ or 2+/ISH+) (n = 72)	HER2 Low (IHC 2+/ISH−, 1+ or 0) (n = 65)
ORR, %	56	42
mPFS, mo	14.1	11.0 月

Outcomes with T-DXd treatment	HER2 QCS+ (n = 40)	HER2 QCS− (n = 25)
ORR, %	52	24
mPFS, mo	14.5	8.6

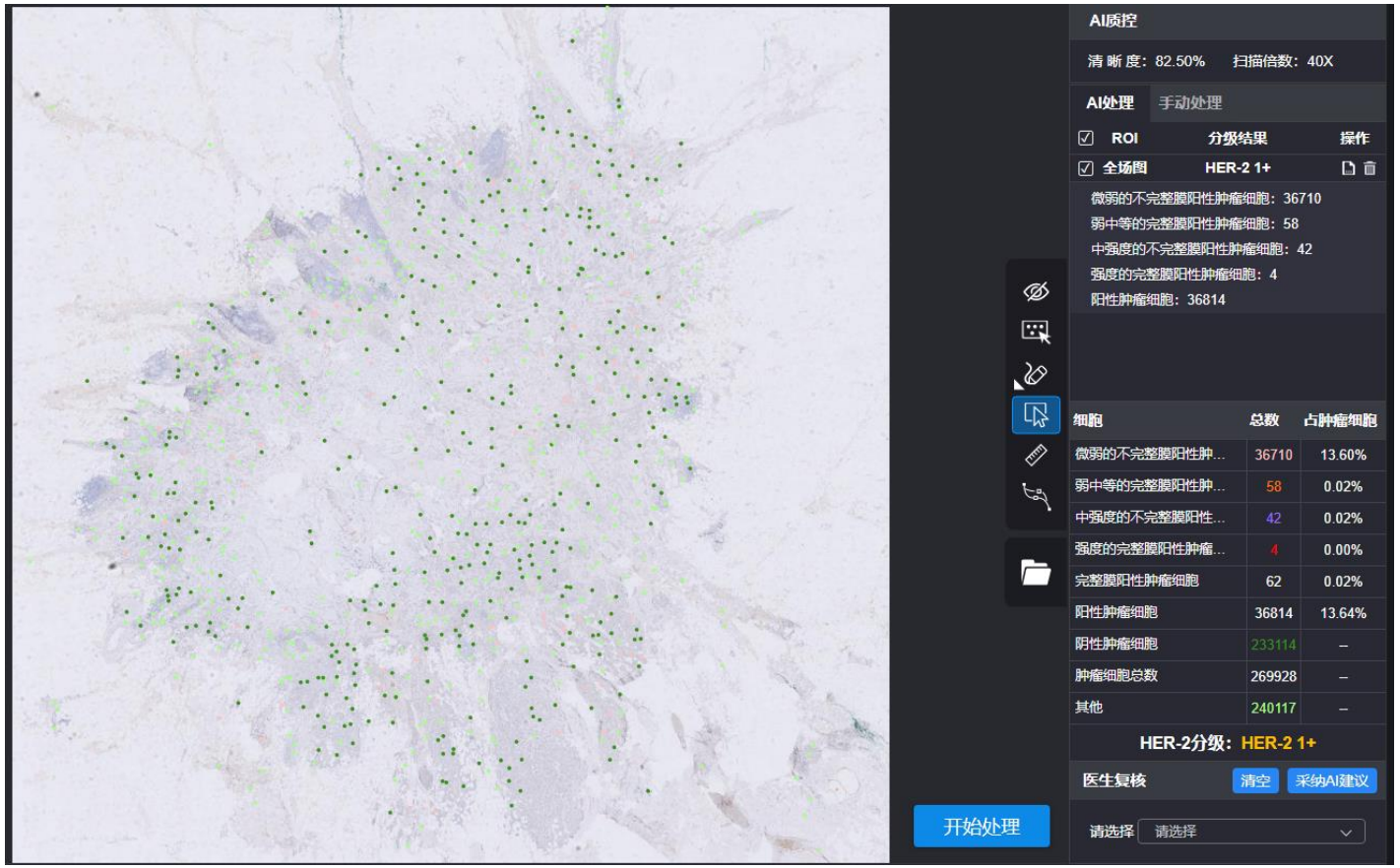
Using QCS scoring, the same population is divided into high-QCS group and low-QCS group: mPFS in the high-QCS group is increased to 14.5 months, while the mPFS in the low-QCS group is only 8.6 months

Retrospective analysis of DS8201-A-J101 study: Among 65 patients with HER2 low tumors, 42% of patients treated with T-DXd responded, and the median progression-free survival (PFS) was 11 months.

Kapil A, Spitzmüller A, Brieu N,et al. Sci Rep. 2024 May 27;14(1) 12129.
Gustavson M, et al., Cancer Research 81:PD6-01-PD6-01, 2021

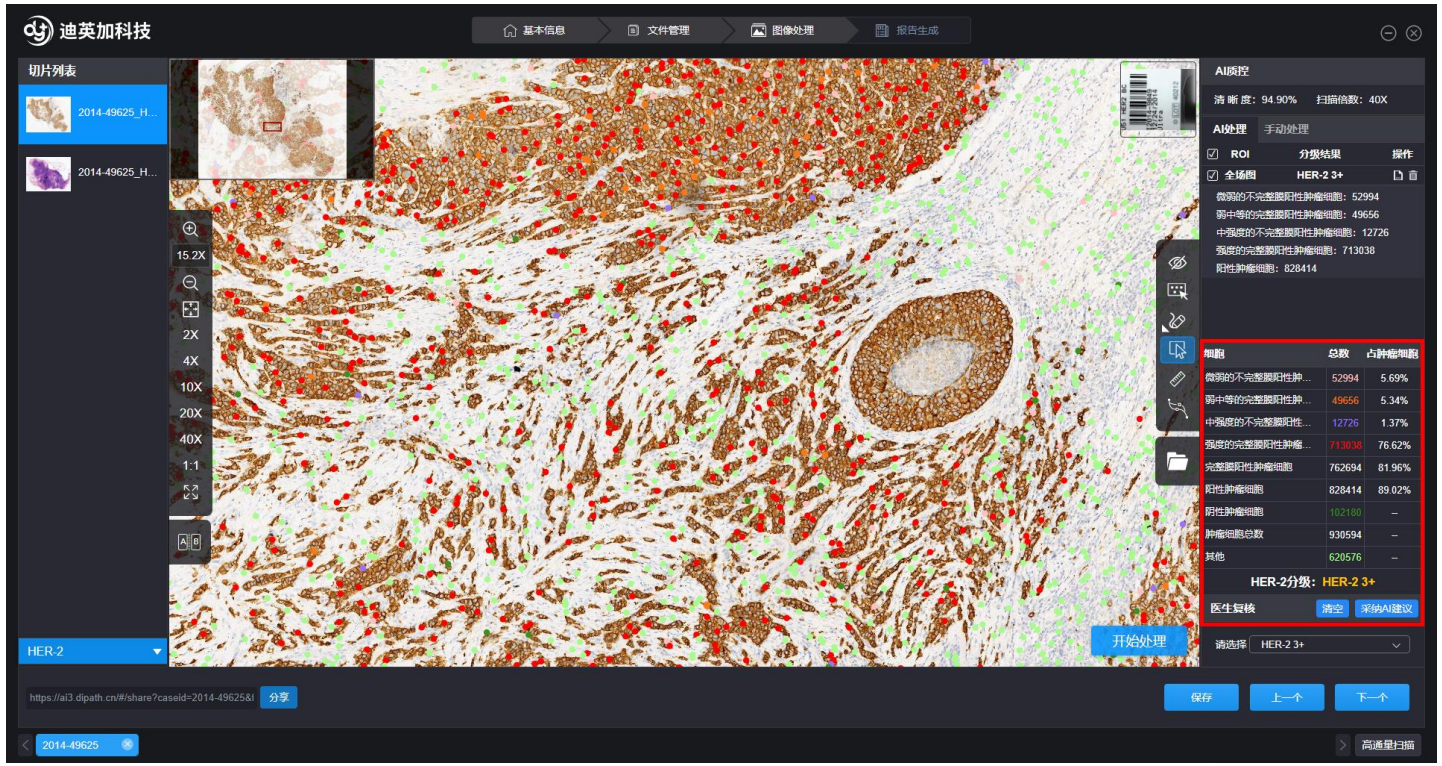
Pathologists’ expectations for HER2 AI adoption in routine clinical practice

Total tumor cells counted: 270,000
Tumor cells with weak, incomplete membrane staining: 35,600 (13.2%)
• **Overall Assessment: HER2 1+**

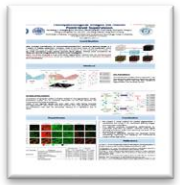


- Familiar whole-slide image scenarios
- based on only HER2 staining (without additional myoepithelial stained sections for AI analysis)
- Should not consume too much of the pathologist's overall diagnostic time
- Seamless integration with pathology information system
- Detect subtle or faint staining that is easily missed by pathologists under microscope
- However, it should not misinterpret background or non-specific staining as membrane staining
- Accurately identify carcinoma in situ, stromal cells, and normal breast tissue

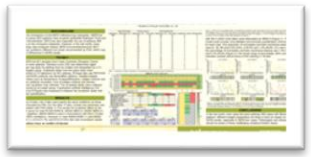
HER2 AI algorithm used in FUSCC



MICCAI 2023 poster Algorithm validation



USCAP 2024 poster Exploratory evidence (AI as analysis tool)



USCAP 2024 oral presentation Clinical validation (FUSCC)

SABCS 2023 poster Analytical validation

USCAP 2024 poster Exploratory evidence (AI as analysis tool)

USCAP 2024 oral presentation Clinical validation (10 sites)

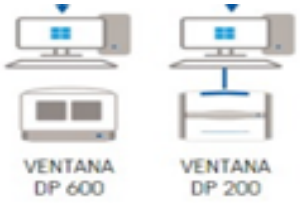
Automatically analyses the digital slides of the entire breast cancer, **excluding intraductal carcinoma and other non-tumour cells**

Classifies and counts tumour cells with different membrane staining integrity and expression intensity

Provides objective diagnostic evidence for HER2 grading by calculating the number and proportion of various types of staining pattern, especially for HER2 IHC 0 and 1+

AI, artificial intelligence; FUSCC, Fudan University Shanghai Cancer Centre; IHC, immunohistochemistry; MICCAI, International Conference on Medical Image Computing and Computer Assisted Intervention; SABCS, San Antonio Breast Cancer Symposium; USCAP, United States and Canadian Academy of Pathology.

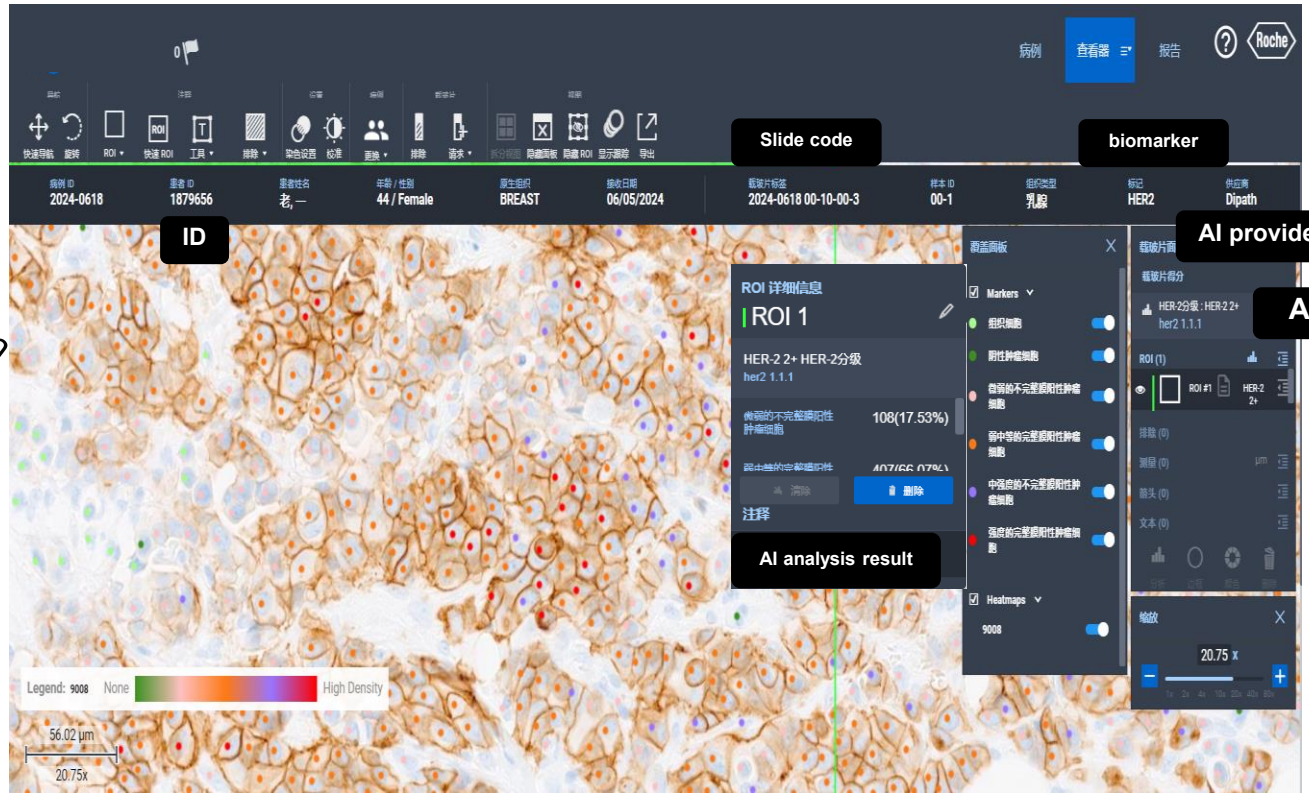
Integration of HER2 AI into part of the workflow in FUSCC

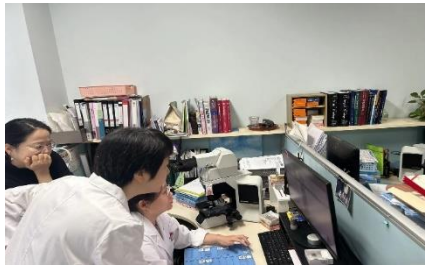


Scanner

Local image system (LIS)

LIS interface and algorithm integration





Clinical adoption scenarios

Roche Navify Digital Pathology hub

HER2 AI from Dipath is integrated

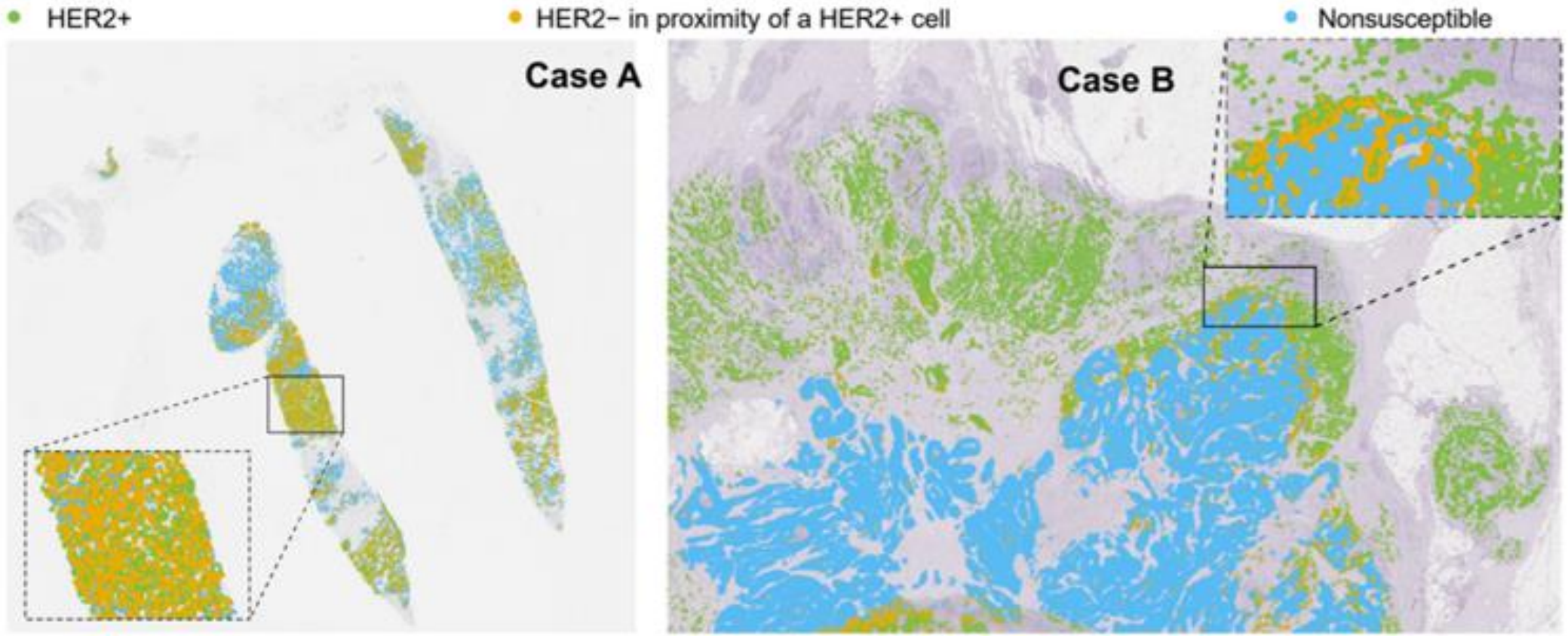
Automatic full slide or selected area delineation, overlay layer to identify tumour cells, and count and calculate percentages

AI, artificial intelligence; FUSCC, Fudan University Shanghai Cancer Centre; LIS, local image system.

Spatial heterogeneity of HER2 status are associated with ADC drug efficacy

Case A: Strongly mixed populations throughout the whole tissue section

Case B: Two clearly separated populations recognisable with SPS capturing only a few cells along the border between the two populations as potentially ADC susceptible



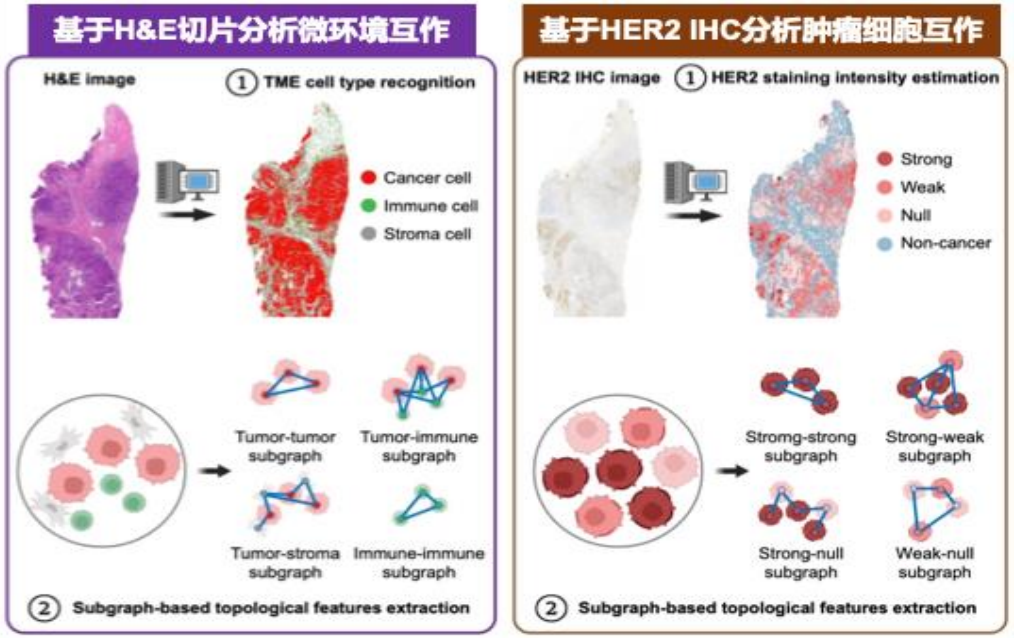
The two cases have similar numbers of HER2-expressing cells (27.3% and 26.6% in case A and case B, respectively), but very different numbers of potentially ADC-susceptible cells (68.0% vs 31.7%)

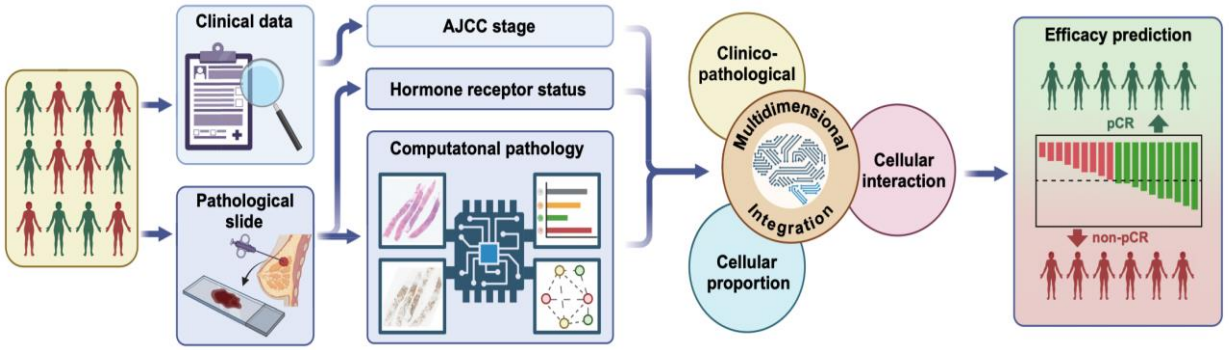
ADC, antibody drug conjugate; SPS, spatial proximity score.

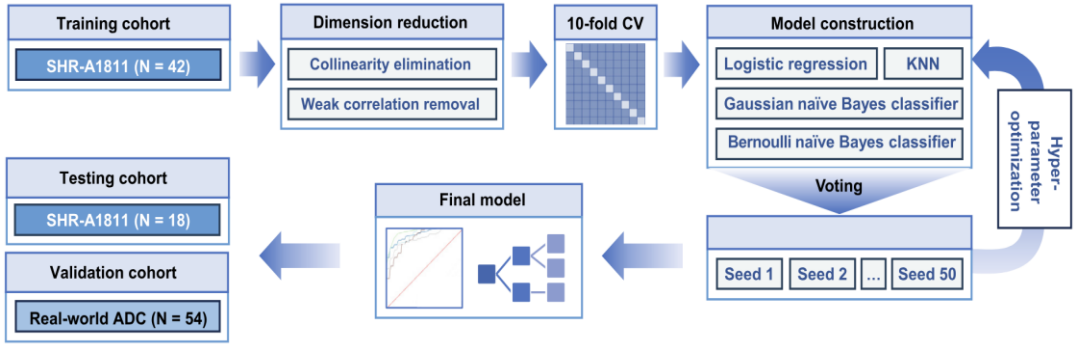
Spitzmüller A, et al. *Cancer Res*. 2023;83(5_Supplement):P6–04–03.

Artificial intelligence digital pathology models predict anti-HER2 targeted therapy-a clinically accessible and highly interpretable predictive model for anti-HER2 ADC efficacy.

- **Clinical accessibility:** Utilizes initial clinical data and needle biopsy digital pathology slides.
- **Strong interpretability:** Model variables consist of AI-extracted features from paired H&E and HER2 IHC stained slides.
- **Predictive performance:** Demonstrates high efficacy for SHR-A1811 and T-DXd but poor for PCbHP, reflecting the model's specificity for ADCs.
- **Technical pathway:** AI-based pathology feature extraction + machine learning modeling + validation across multiple external cohorts.







Cancer Cell. 2025 Apr 4:S1535-6108(25)00118-7.

Summary

Challenges for HER2 low/ultra-low testing in breast cancer

- **There are difficulties in accurately distinguishing HER2 1+, HER2 ultralow and HER2-null (especially near the critical value).**
- **The expression rates of HER2-low by different antibodies and platforms are different. Which antibodies tested HER2-low is more relevant to the treatment effect?**
- **The influence of pre-analytical variables (decalcification, sample type, cold ischemic time, fixation time, etc.) on HER2-low/ultra-low testing requires more exploration**
- **The lower limit of HER2 protein expression required for response to ADCs is not yet defined;**

Coping strategies

- Strictly follow standardized testing guidelines.
- Enhance training and external quality assurance.
- Compare antibodies and platforms using real-world treatment data.
- Investigate pre-analytical impacts on HER2-low/ultralow results.
- Incorporate new technologies (AI, quantification, liquid biopsy).
- Strengthen collaboration between clinical and pathology teams.


Thank you