Memorial Sloan-Kettering Cancer Center

29th Annual Seminar in Pathology

Pittsburgh, PA. April 27, 2023 to April 29, 2023

Prognostic and Predictive Markers in Breast Cancer

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Memorial Sloan Kettering Cancer Center

Disclosure

- Advisory faculty, AstraZeneca

Outline

- HER2
- Ki67
- Multigene assays
- PD-L1

HER2

The NEW ENGLAND JOURNAL of MEDICINE

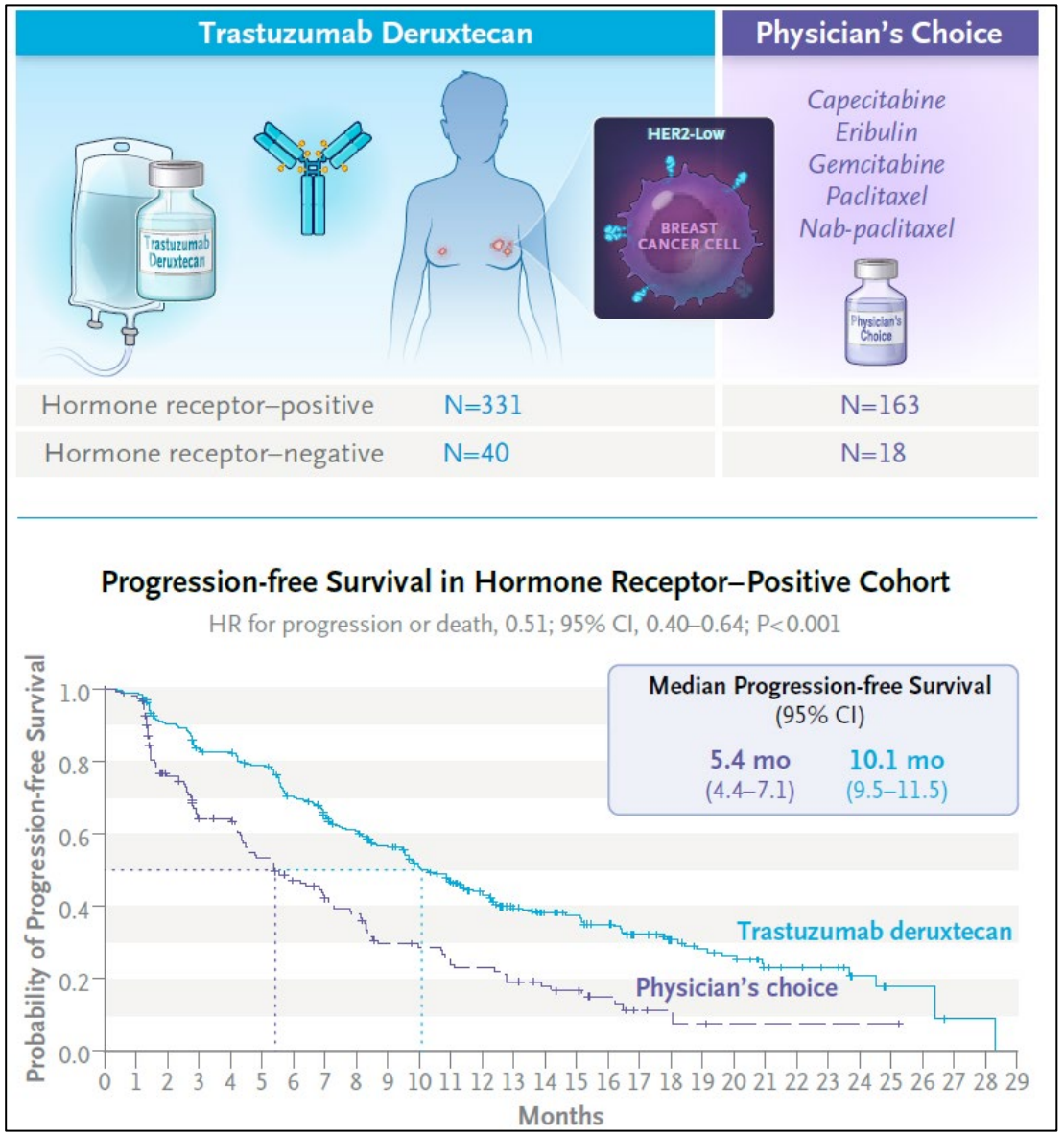
ESTABLISHED IN 1812 JULY 7, 2022 VOL. 387 NO. 1

Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer

S. Modi, W. Jacot, T. Yamashita, J. Sohn, M. Vidal, E. Tokunaga, J. Tsurutani, N.T. Ueno, A. Prat, Y.S. Chae, K.S. Lee, N. Niikura, Y.H. Park, B. Xu, X. Wang, M. Gil-Gil, W. Li, J.-Y. Pierga, S.-A. Im, H.C.F. Moore, H.S. Rugo, R. Yerushalmi, F. Zagouri, A. Gombos, S.-B. Kim, Q. Liu, T. Luo, C. Saura, P. Schmid, T. Sun, D. Gambhire, L. Yung, Y. Wang, J. Singh, P. Vitazka, G. Meinhardt, N. Harbeck, and D.A. Cameron, for the DESTINY-Breast04 Trial Investigators*

Trastuzumab deruxtecan targeting low level of HER2

- Trastuzumab deruxtecan (T-Dxd), an antibody–drug conjugate c(ADC) onsisting of a humanized anti-HER2 monoclonal antibody linked to a topoisomerase I inhibitor
- DESTINY-Breast04: T-DXd doubled progression-free survival in patients with ***HER2-low*** metastatic breast cancer

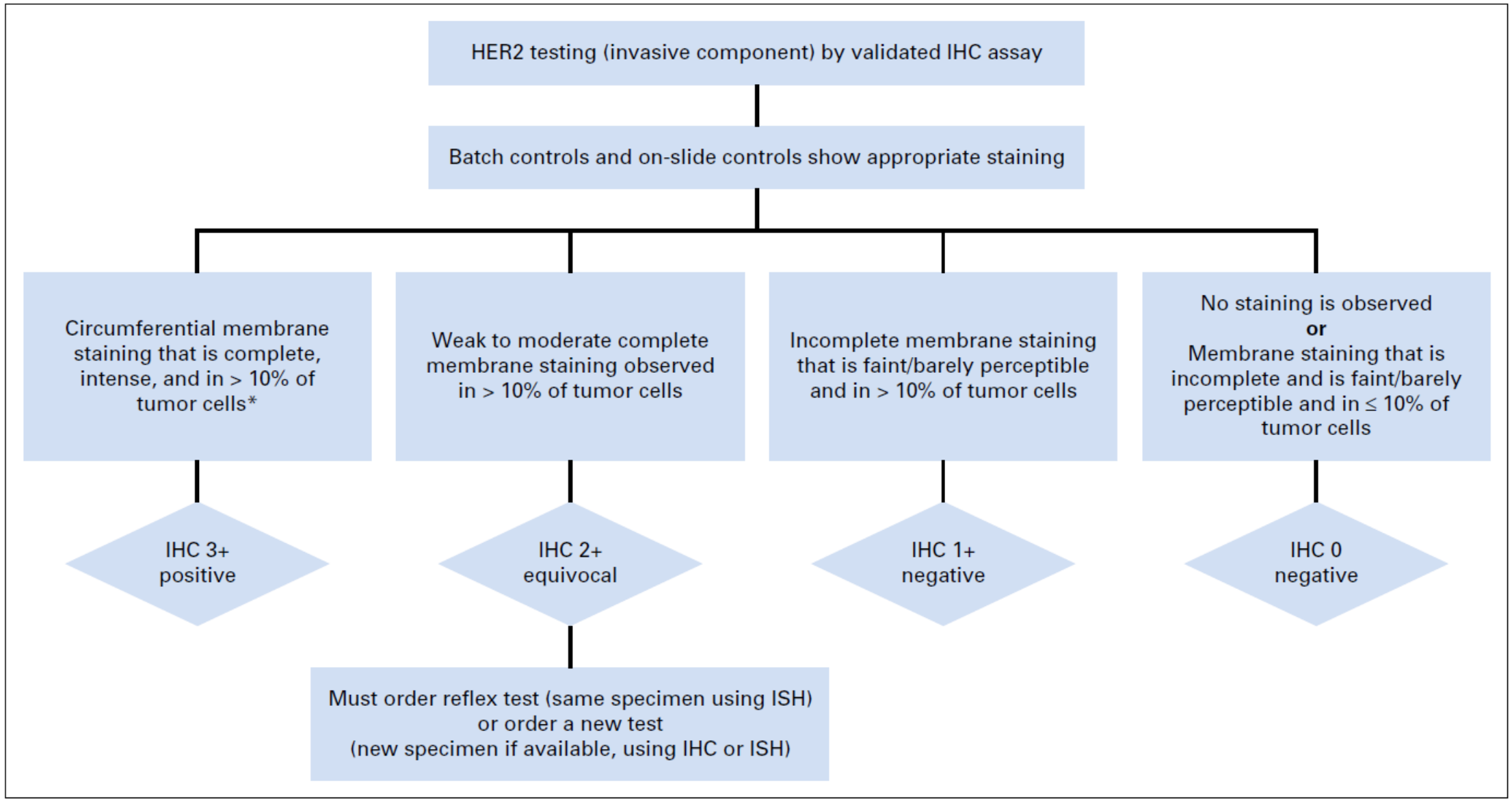


Modi S. et al. *N Engl J Med*. 2022; 387:9-20

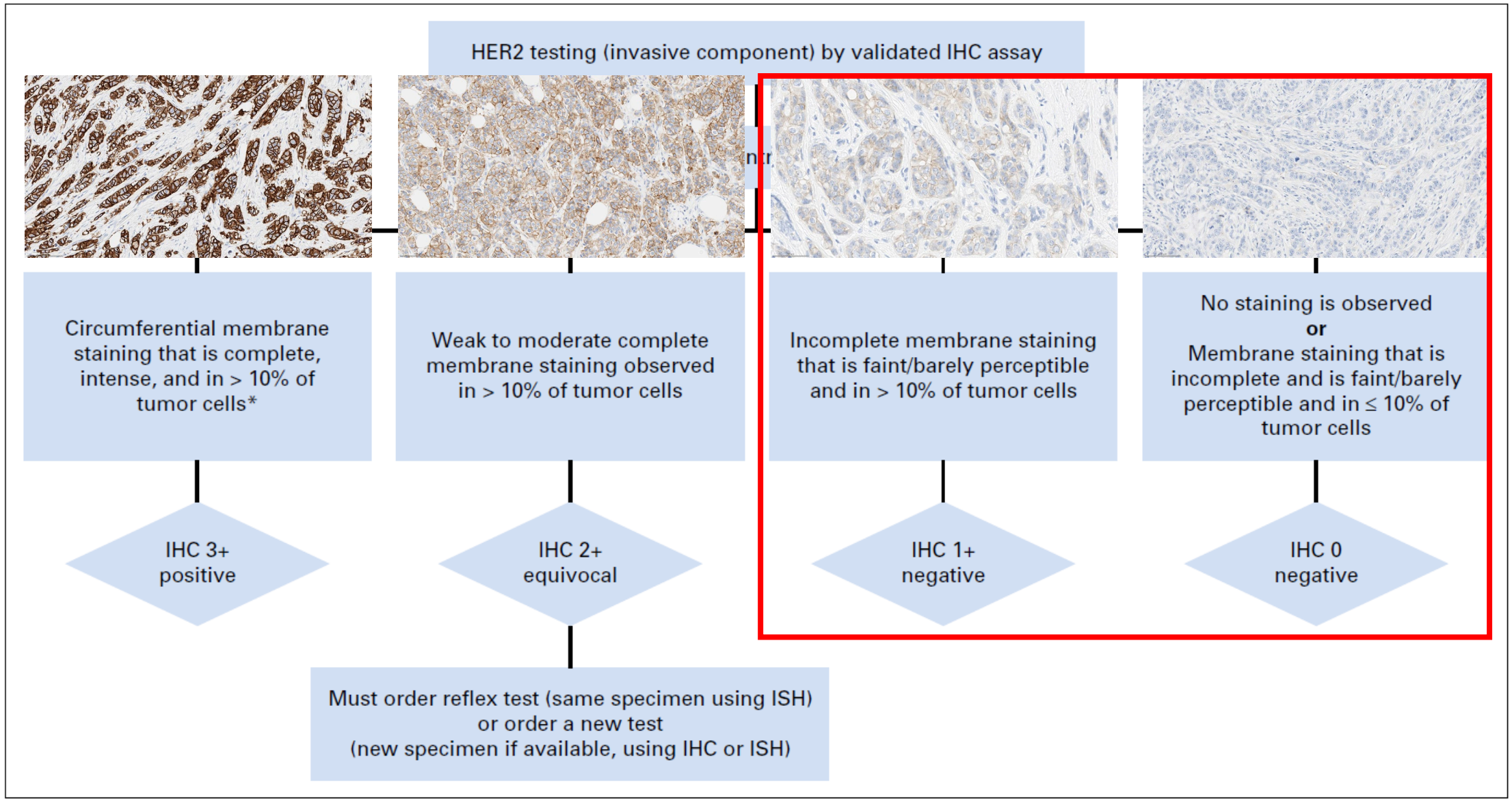
HER2-low

HER2 1+ by IHC

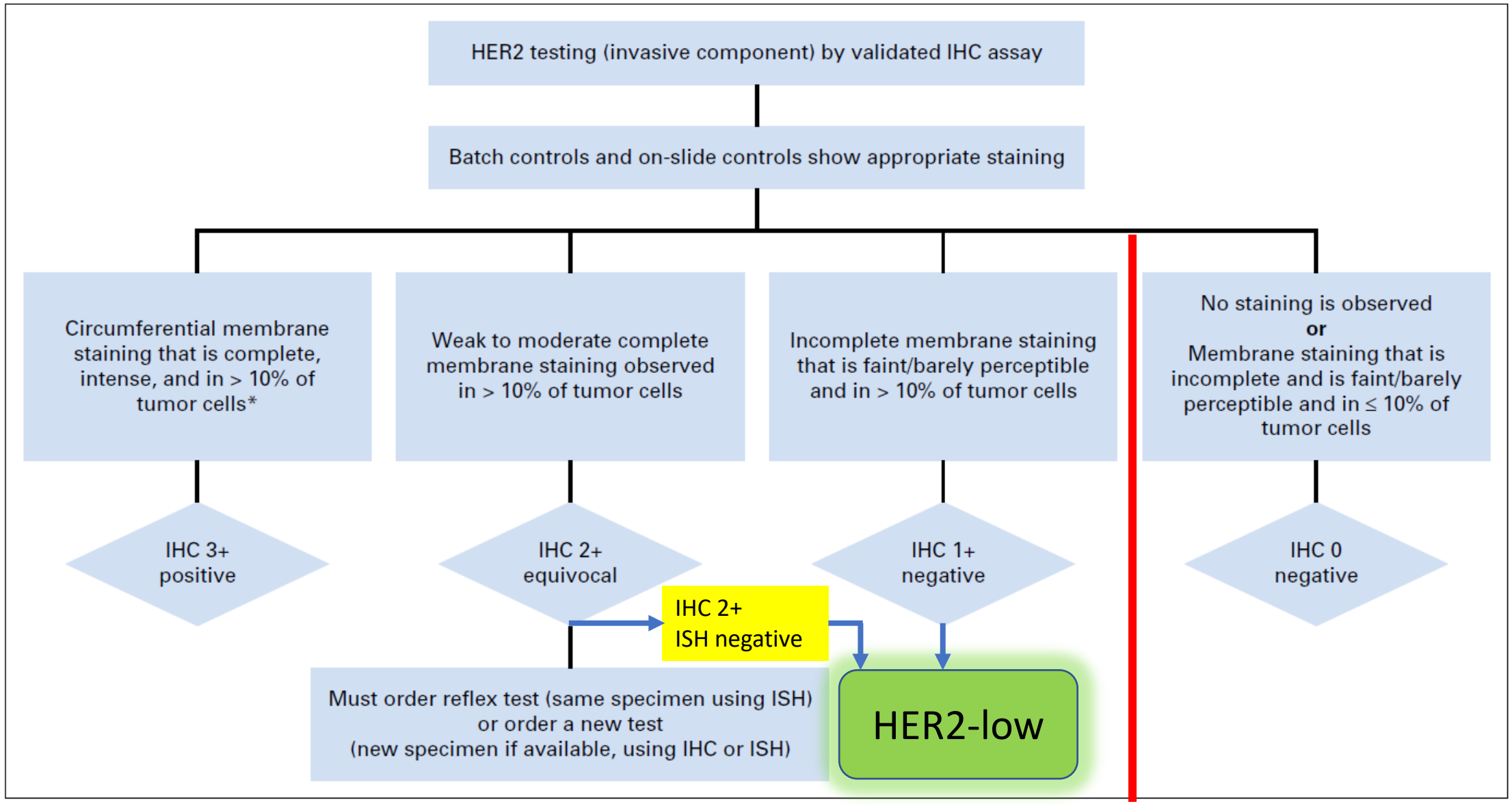
HER2 2+ by IHC,
not amplified by ISH



Wolff AC et al. *J Clin Oncol.* 2018; 36: 2105-2122



Wolff AC et al. *J Clin Oncol.* 2018; 36: 2105-2122


HER2 testing (invasive component) by validated IHC assay
Batch controls and on-slide controls show appropriate staining
Circumferential membrane staining that is complete, intense, and in > 10% of tumor cells*
Weak to moderate complete membrane staining observed in > 10% of tumor cells
Incomplete membrane staining that is faint/barely perceptible and in > 10% of tumor cells
No staining is observed
or
Membrane staining that is incomplete and is faint/barely perceptible and in ≤ 10% of tumor cells
IHC 3+
positive
IHC 2+
equivocal
IHC 1+
negative
IHC 0
negative
IHC 2+
ISH negative
Must order reflex test (same specimen using ISH)
or order a new test
(new specimen if available, using IHC or ISH)
HER2-low

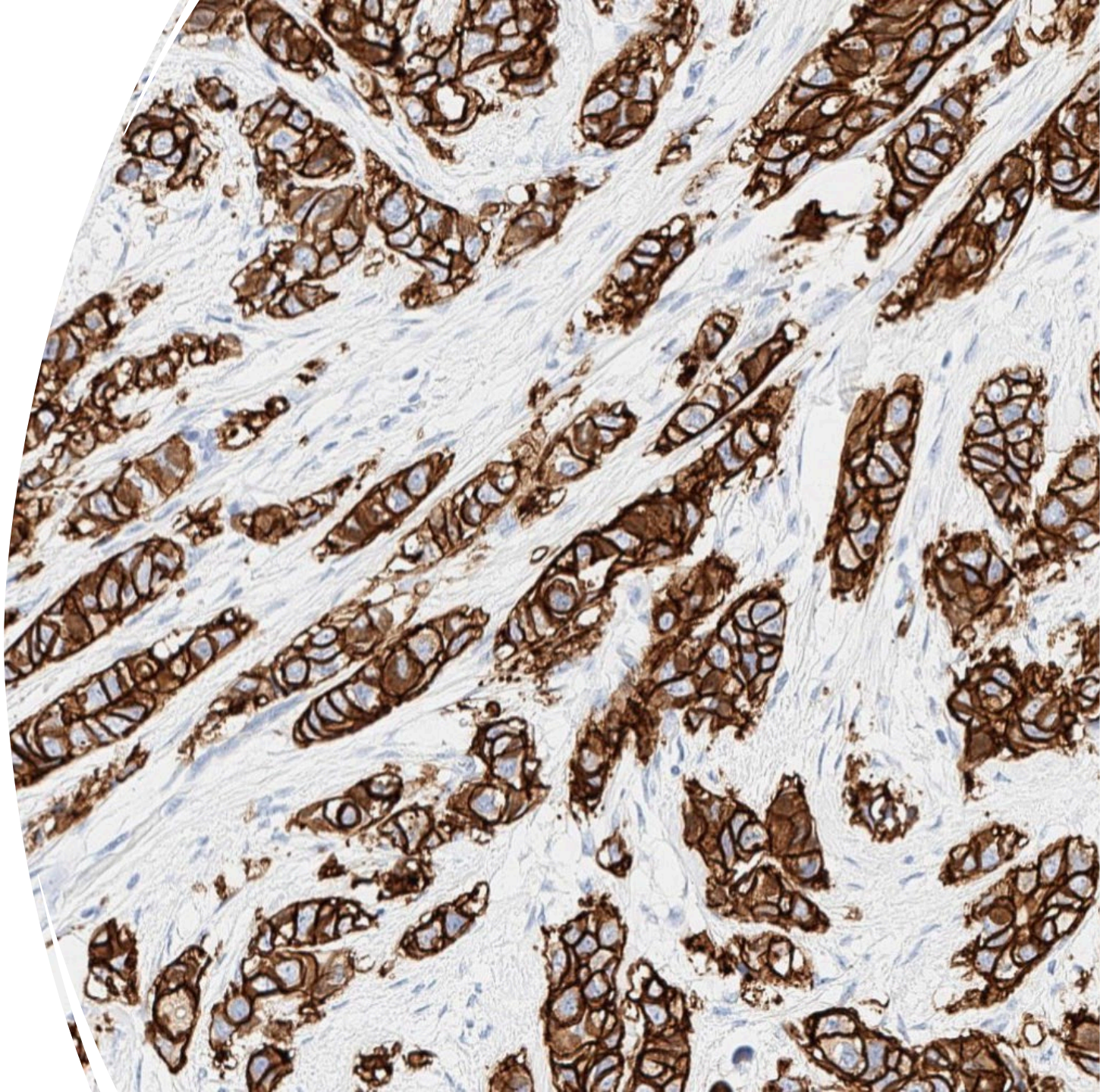
CAP Template for Reporting HER2 IHC in Breast Cancer (March 2023)

- Negative (score 0)
- Negative (score 1+)#
- Equivocal (score 2+)#
- Positive (score 3+)

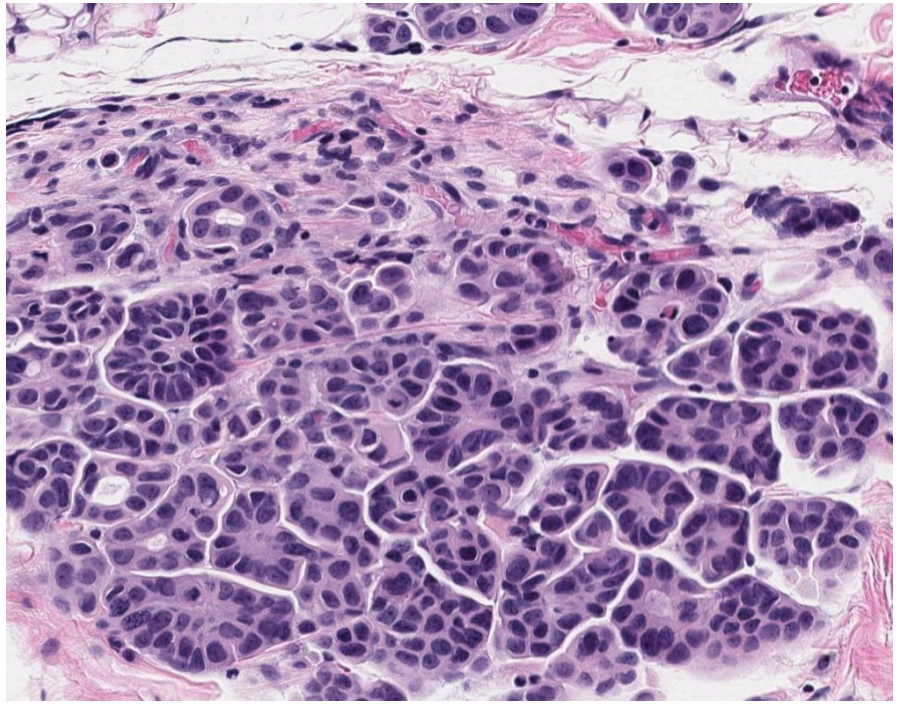
 # Breast cancers with HER2 IHC score 1+ or HER2 IHC score 2+ and a negative ISH result are eligible for clinically appropriate HER2-targeted therapy and may be reported as "HER2 Low".

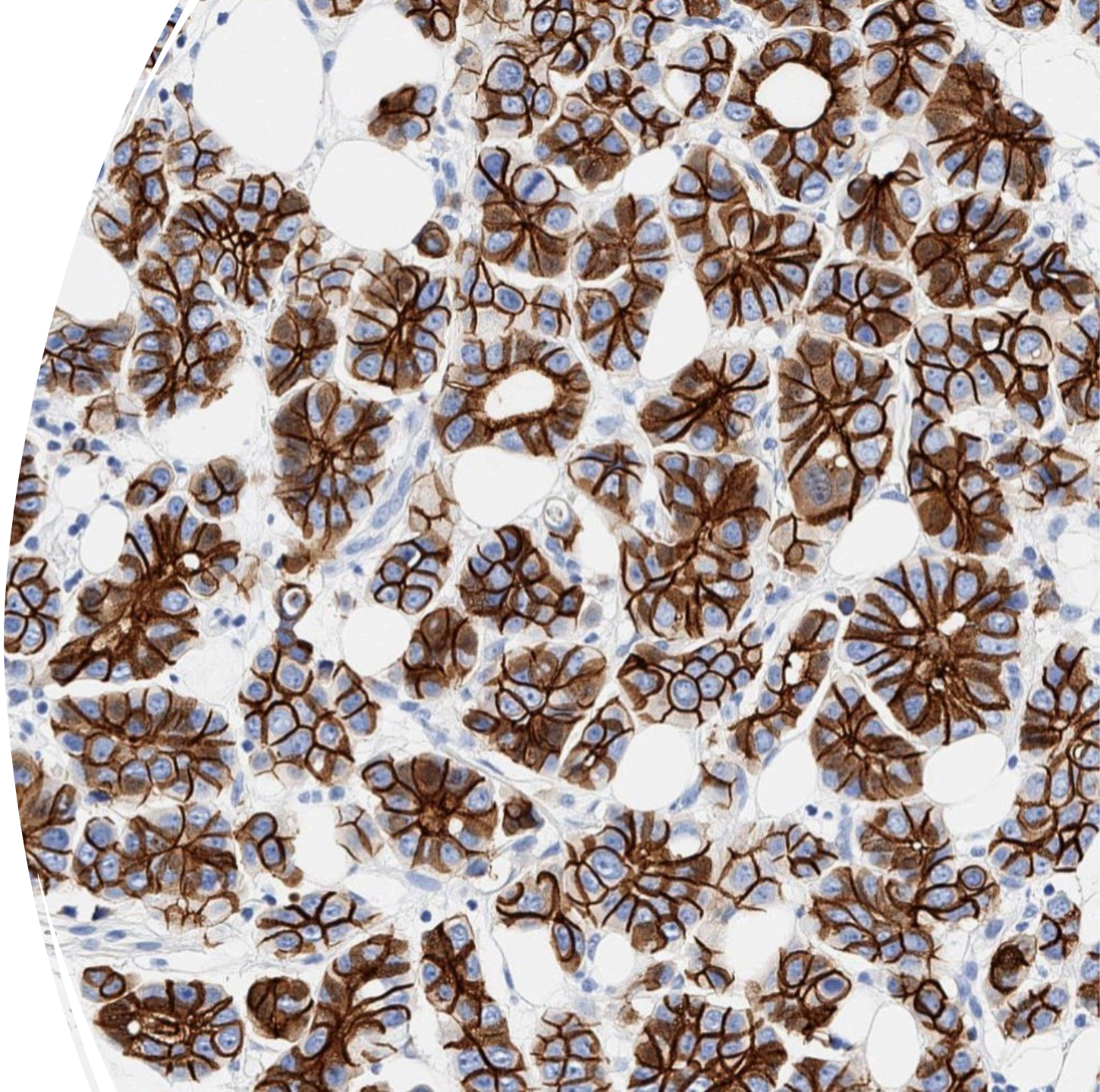
HER2 IHC 3+

- Membrane staining
  - Pattern: complete
  - Intensity: strong
  - Percentage: >10% of tumor cells



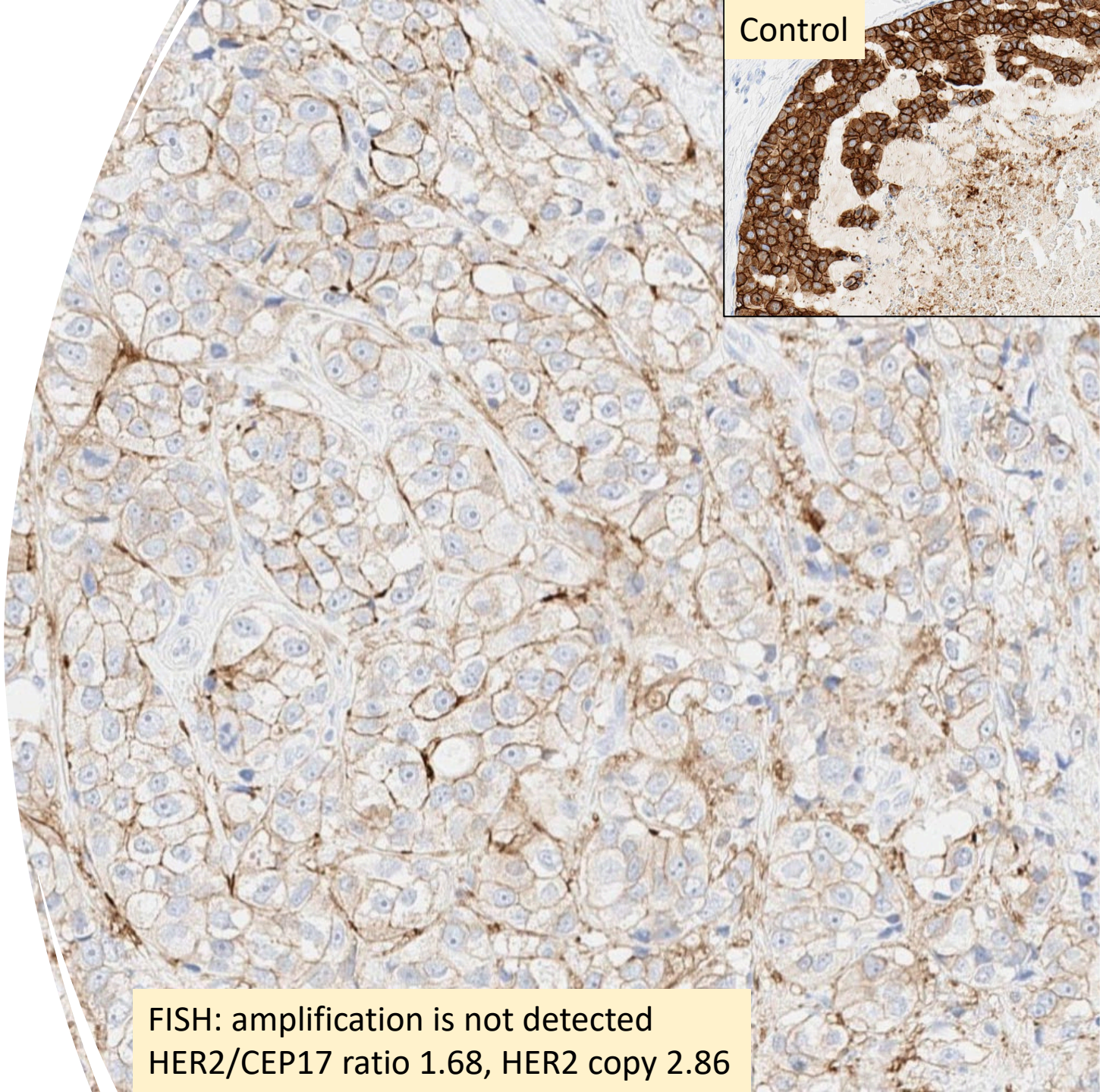
Basolateral staining in micropapillary carcinoma





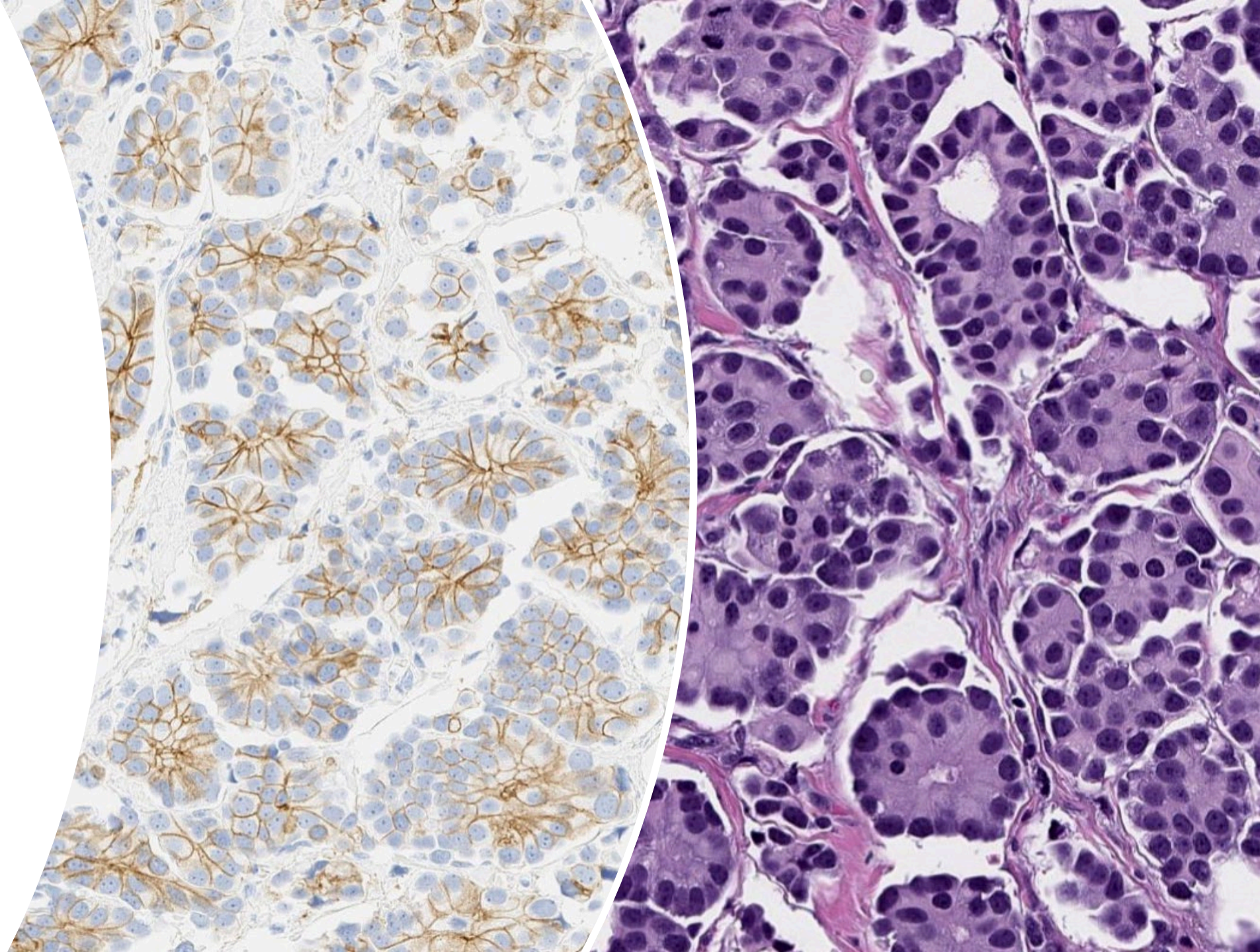
HER2 IHC 2+

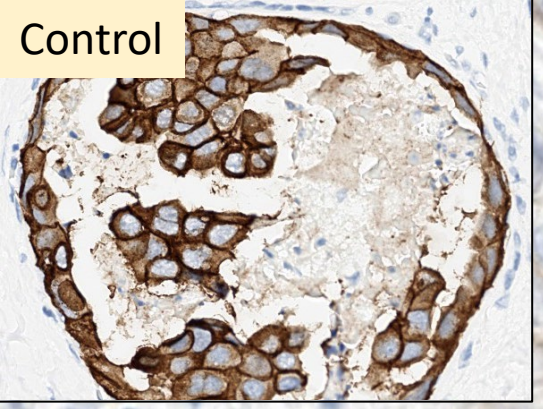
- Membrane staining
  - Pattern: complete
  - *Intensity: weak-moderate*
  - Percentage: >10% of tumor cells



Micropapillary carcinoma

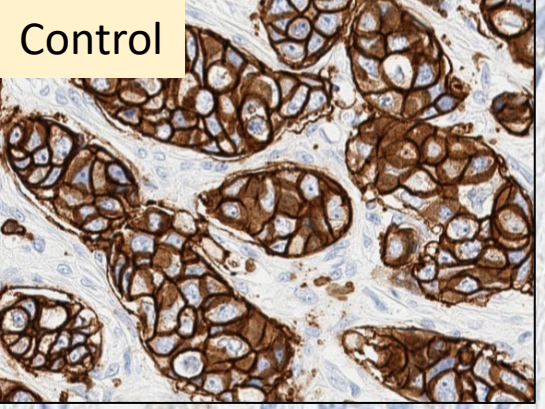
- Basolateral staining
- Moderate intensity
  - IHC equivocal (2+)
- HER2 FISH: amplification is DETECTED
- HER2/ CEP 17 ratio: 2.5
- HER2 copy number: 5.1





HER2 IHC 1+

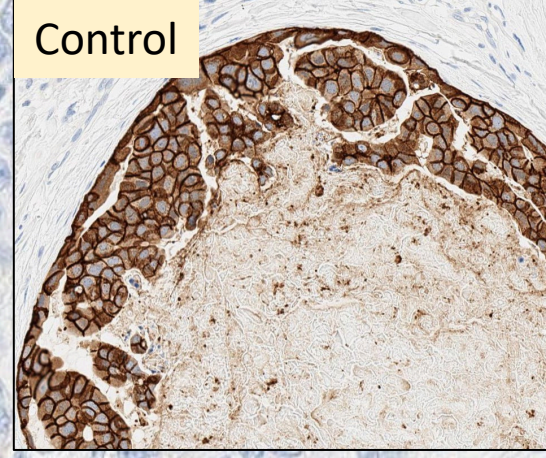
- Membrane staining
  - *Pattern: incomplete*
  - *Intensity: weak*
  - Percentage: >10% of tumor cells



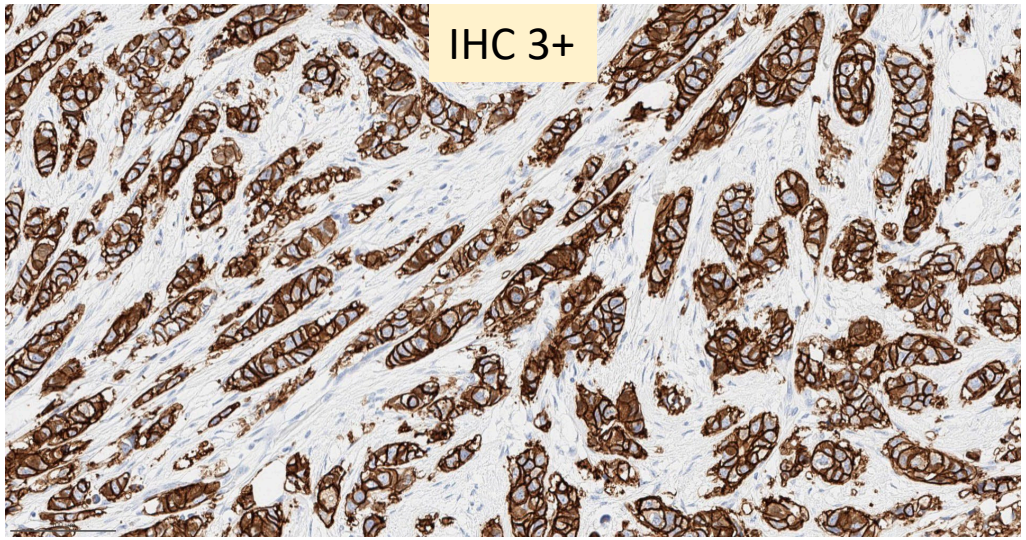
HER2 IHC 0

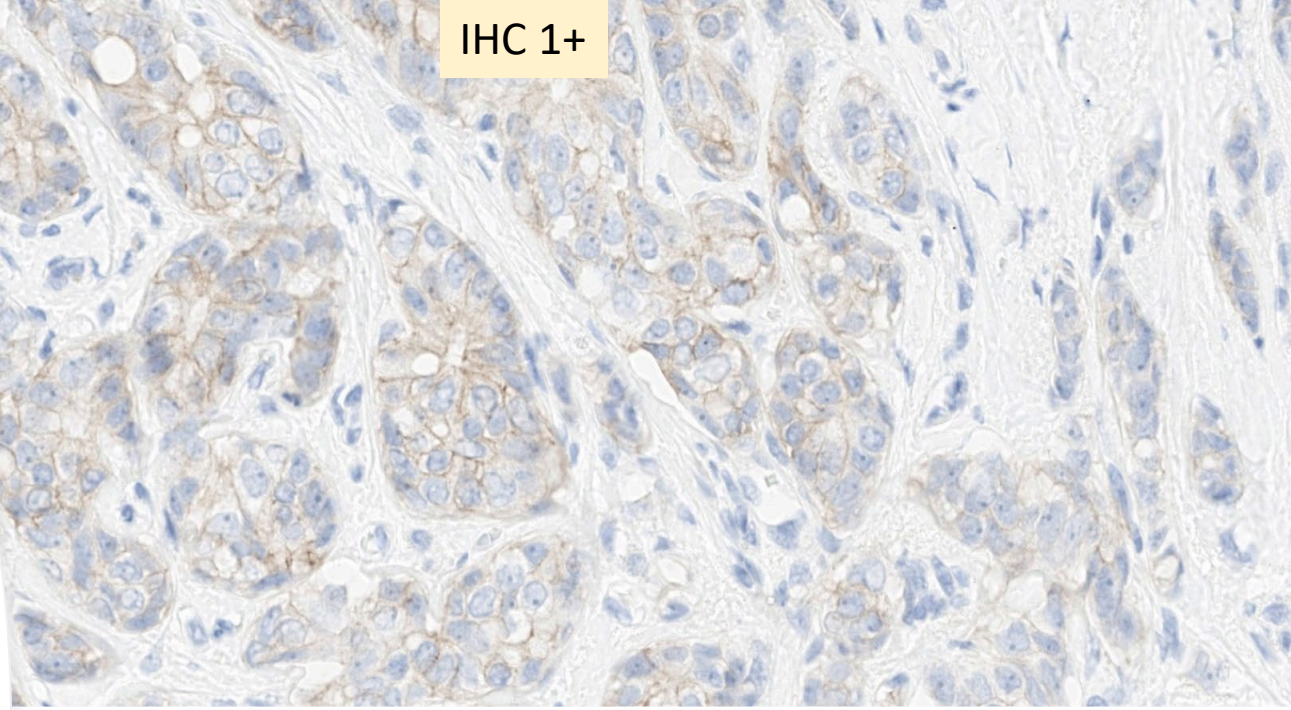
- Membrane staining
  - *Pattern: incomplete*
  - *Intensity: weak*
  - *Percentage: ≤10% of tumor cells*

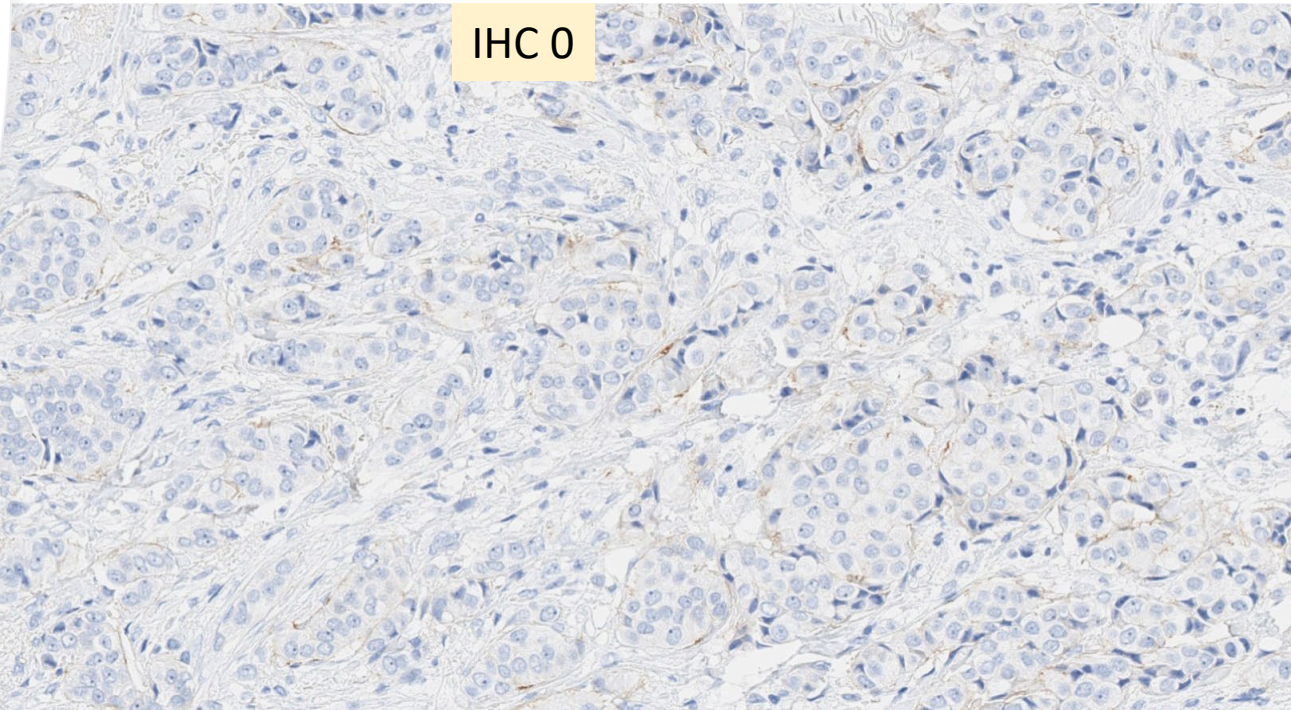
HER2 IHC 0
(No staining)

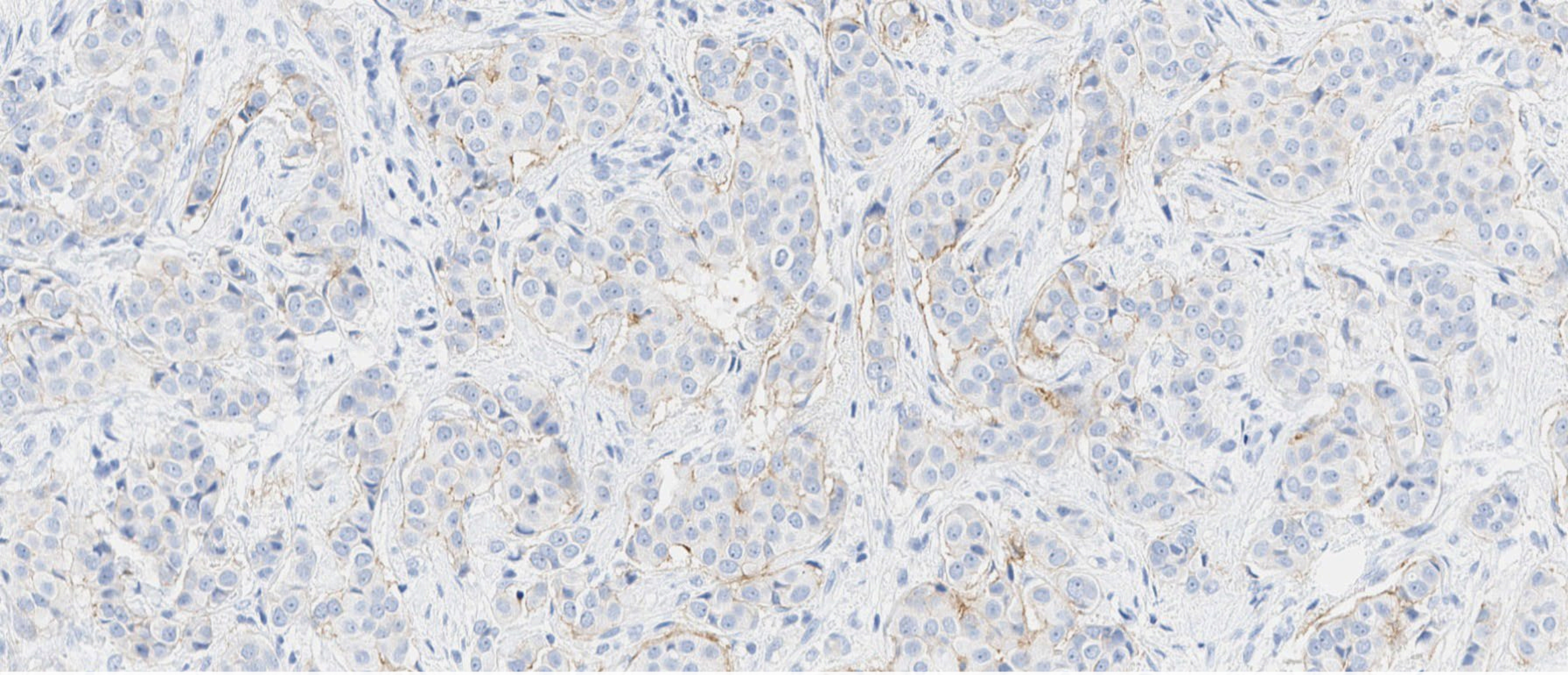


HER2 low assessment by IHC: challenges in the interpretation

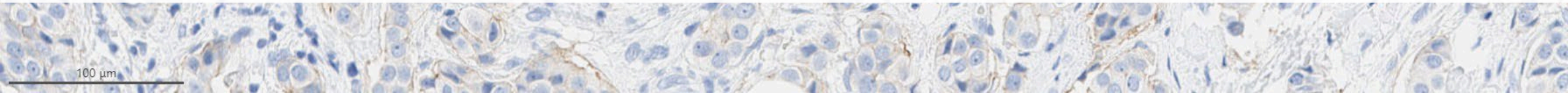


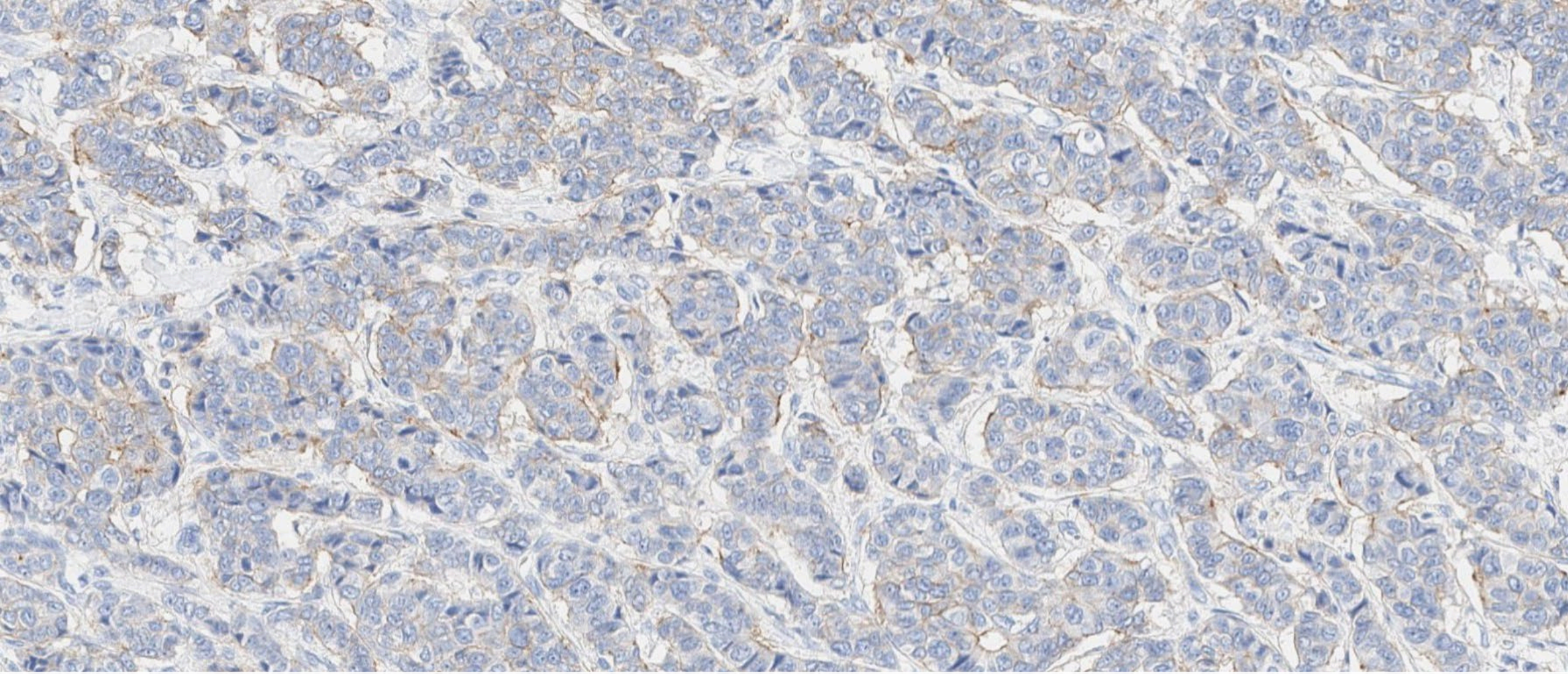




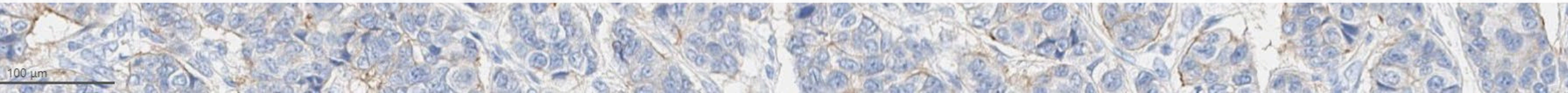


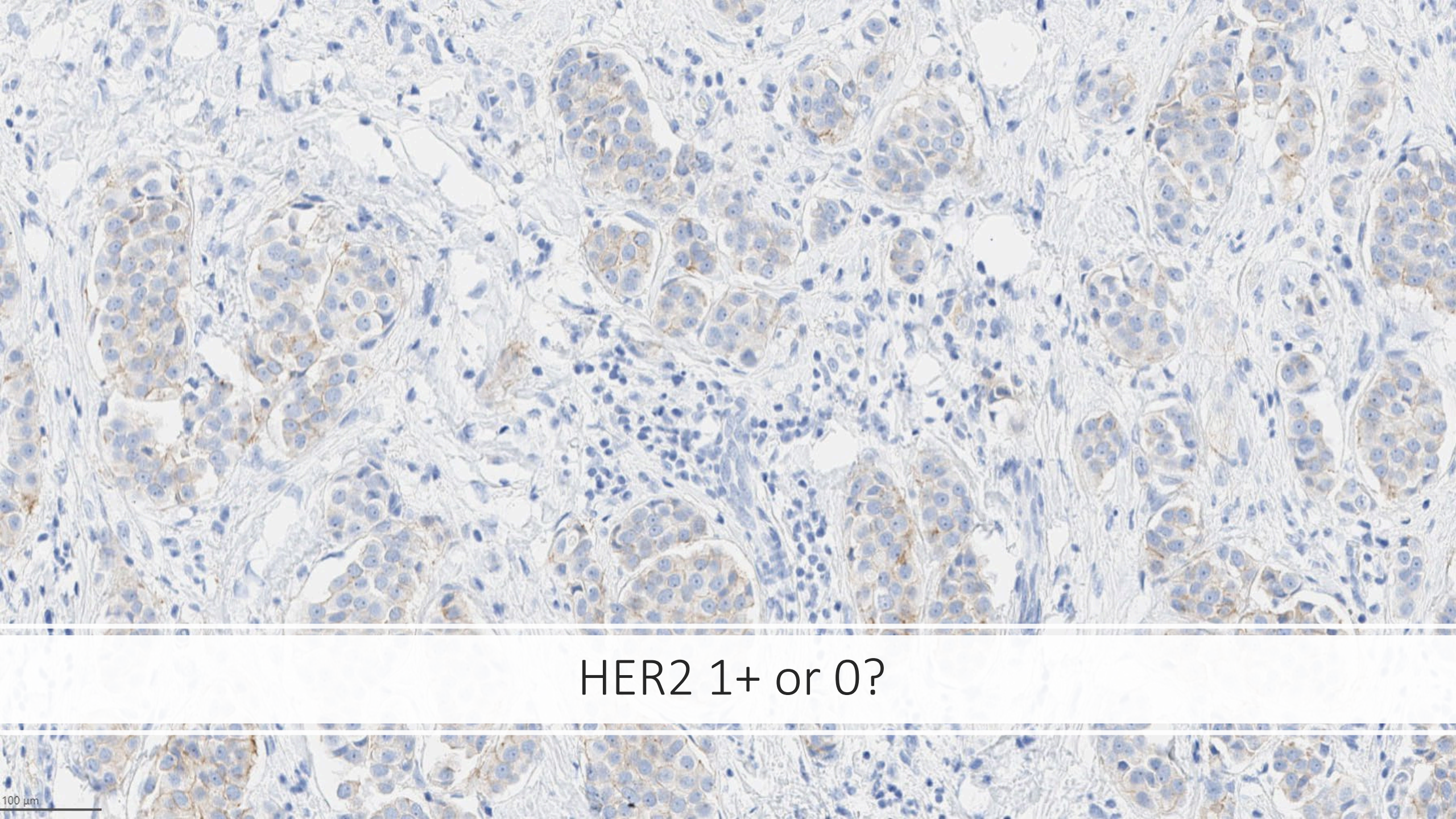
HER2 1+ or 0?





HER2 1+ or 0?





HER2 1+ or 0?

DESTINY-Breast06

Study of trastuzumab deruxtecan (T-Dxd) vs investigator's choice chemotherapy in HER2-low, hormone receptor positive, metastatic breast cancer

Eligible patients

HER2-low:	*HER2-ultra low:*
IHC2+/ISH- and IHC 1+	*HER2 IHC >0 <1+ expression*

https://clinicaltrials.gov/ct2/show/NCT04494425. Estimated primary completion date: July 31, 2023

ASCO/CAP guideline: pre-analytical standardization Optimal tissue handling requirements

Time from tissue acquisition to fixation should be as short as possible. Cold ischemia time <1 hour

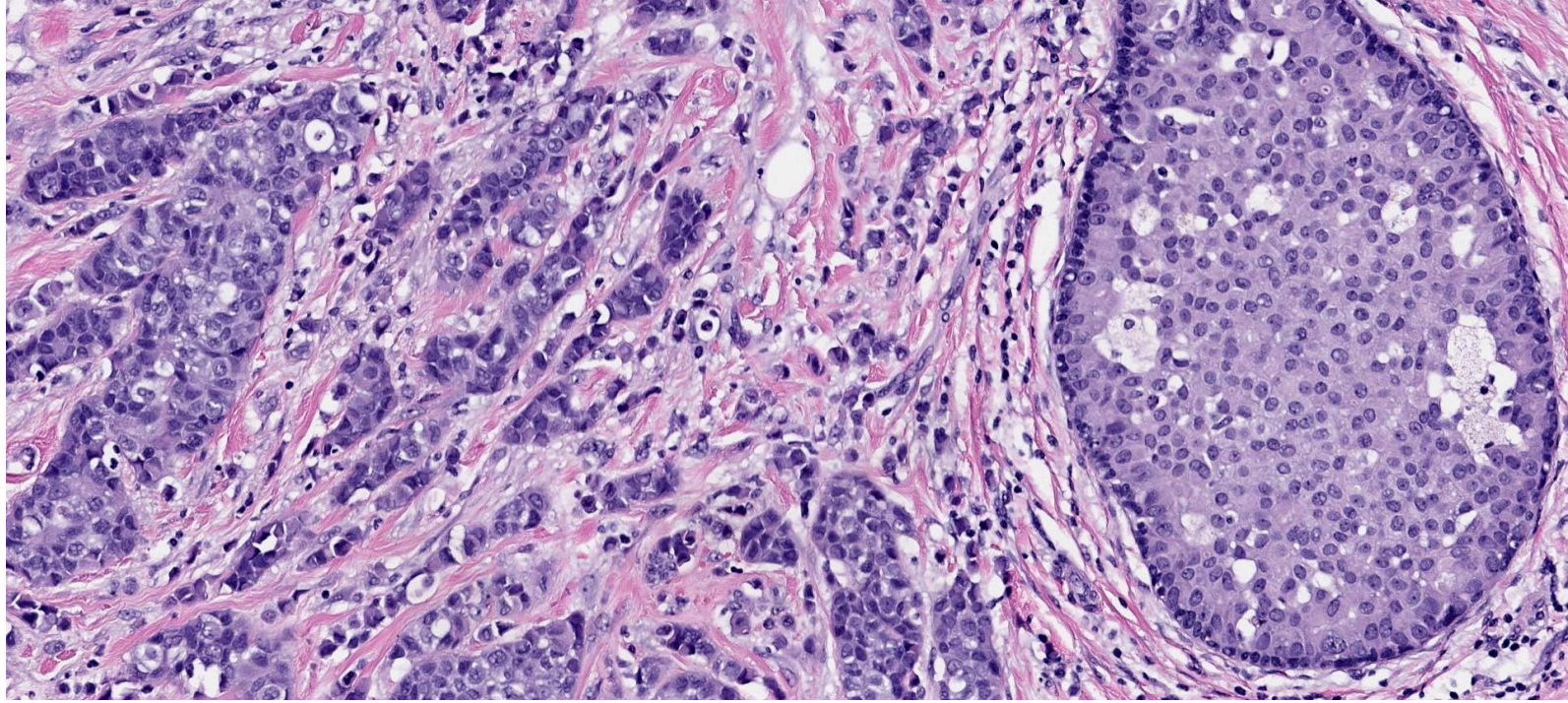
Samples are fixed in 10% neutral buffered formalin for 6-72 hours

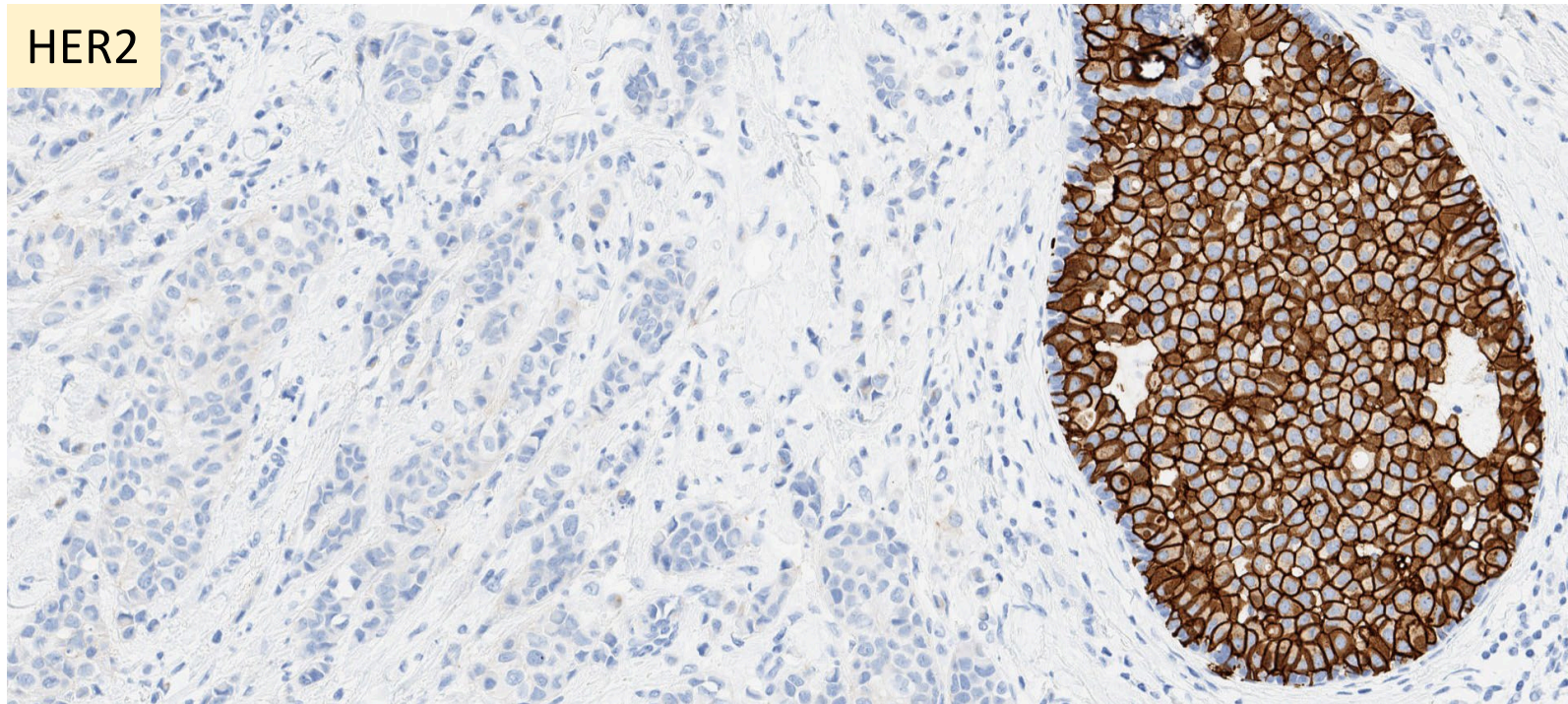
Cytology specimens must be fixed in formalin

Samples should be sliced at 5- to 10-mm intervals and placed in sufficient volume of neutral buffered formalin

Review the corresponding H&E stain

- Discordant HER2 expression between invasive and in situ carcinoma
- HER2 expression in the in situ component should be excluded from the interpretation





ASCO/CAP guideline: specimens to be tested

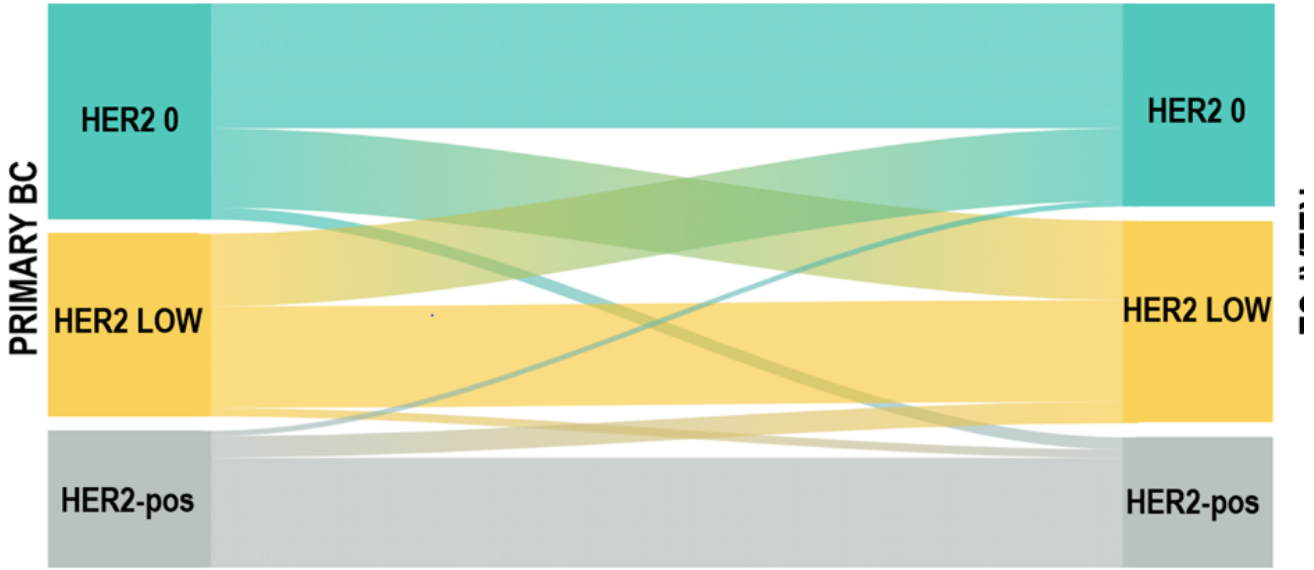
HER2 testing is recommended for primary, recurrent and metastatic tumors

Patients who develop metastatic disease must have a HER2 test performed in a metastatic site, if tissue sample is available

Wolff AC et al. *J Clin Oncol.* 2018; 36: 2105-2122

HER2 evolution from primary breast cancer to recurrence

- Patients with matched primary and recurrent breast cancer samples (n=547)
- HER2 was evaluated according to ASCO/CAP recommendations
- The overall rate of HER2 discordance was 38.0% (*n* = 208), mostly driven by cases switching to or from HER2-low expression
  - Conversion from HER2-0 to HER2-low: 15.2%
  - Conversion from HER2-low to HER2-0 : 14.1%

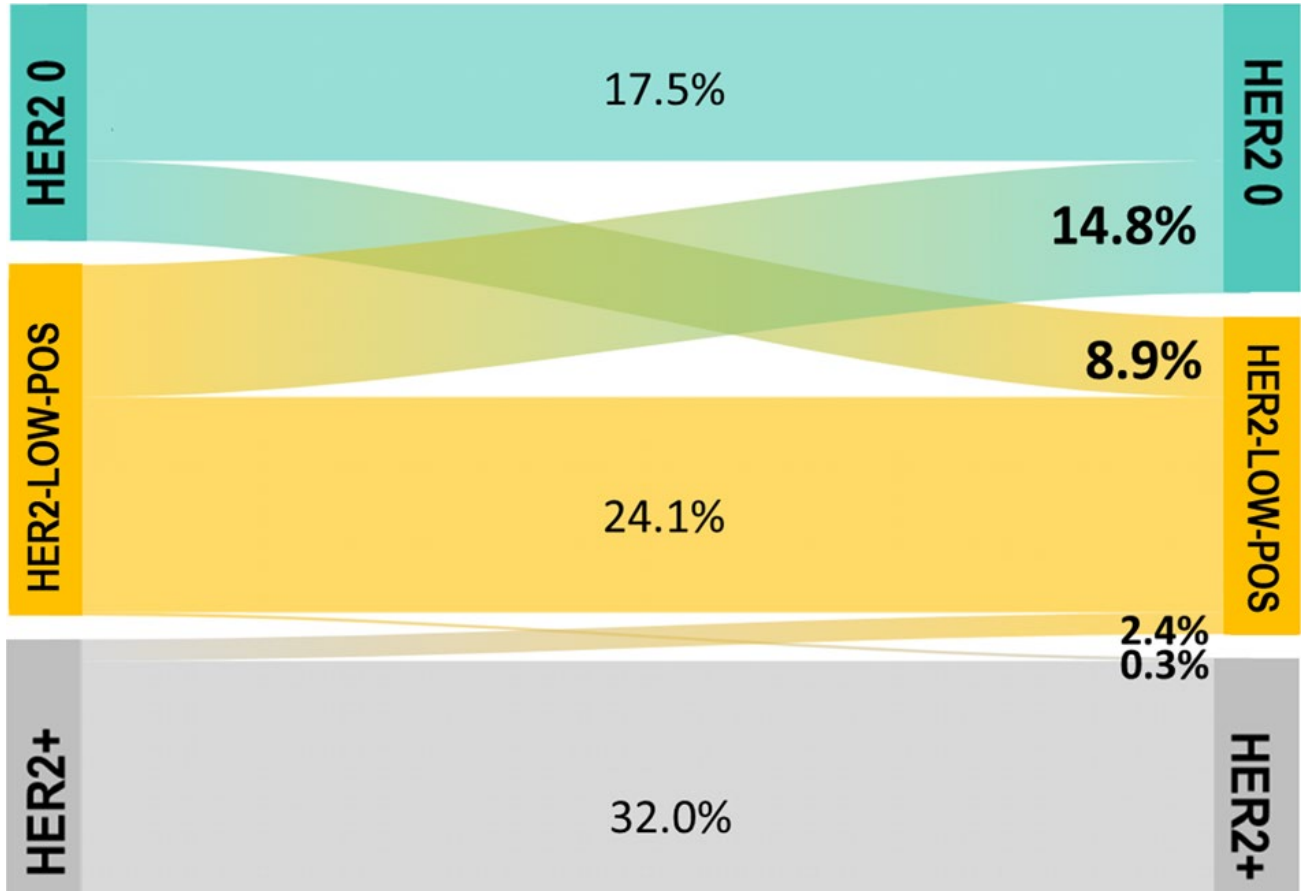


		HER2 recurrence/metastasis N,%			Total
		0	Low	Positive	
HER2 primary BC N,%	0	132 (24.1)	83 (15.2)	13 (2.4)	228 (41.7)
	Low	77 (14.1)	101 (18.5)	9 (1.6)	187 (34.2)
	Positive	6 (1.1)	20 (3.7)	106 (19.4)	132 (24.1)
Total		215 (39.3)	204 (37.3)	128 (23.4)	547 (100)

Miglietta F. et al. NPJ Breast Cancer. 2021; 7:137

HER2 expression: from pre-treatment biopsy to residual tumor post neoadjuvant treatment

- In patients with residual disease after neoadjuvant chemotherapy, the overall rate of HER2 discordance was 26.4%, mostly represented by cases switching between HER2 0 and HER2-low
- Among patients with HER2-0 on baseline biopsy, 33.8% (*n* = 26) experienced a conversion to HER2-low
- Among patients with HER2-low-positive breast cancer, 37.7% (*n* = 43) showed a conversion to HER2 0



Miglietta F. et al. NPJ Breast Cancer. 2022; 8:66

Companion diagnostic assay for HER2-low

HER2 status was assessed by a central laboratory in DESTINY-Breast04

Companion diagnostic assay

- FDA approved companion diagnostic assay: *PATHWAY anti-HER2 (4B5) assay*

Tumor samples

- Archived or recent tumor sample
- Primary tumor or metastatic sites
- *Exclusion: Cytology and decalcified bone metastases*

Modi S. et al. *N Engl J Med*. 2022; 387:9-20

HercepTest and PATHWAY 4B5 — inter-assay concordance

- Complete concordance: 69.7%
- Discordant scores were mainly associated with the PATHWAY 4B5 IHC 0 and IHC 1 +
- Higher HER2 scoring using the HercepTest

		PATHWAY 4B5				
		0	1+	2+	3+	Total
HercepTest (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

Rüschoff J. et al. *Virchows Arch*. 2022; 481:685-694

Ki67

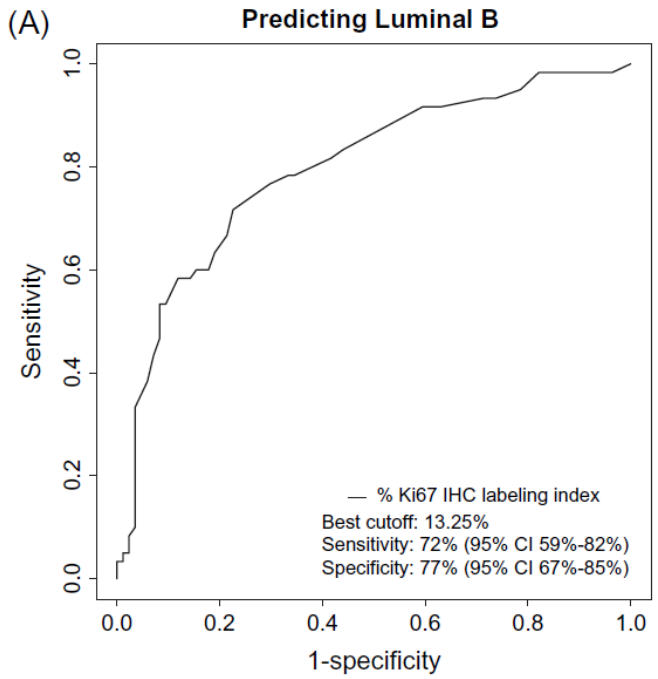
Ki67 as a prognostic marker

Clinical utility is limited

Lack of reproducibility (especially between different laboratories)

Lack of standardized cut-off

Immunohistochemistry surrogate to distinguish between luminal A and luminal B breast caner subtypes



- ROC analysis of 144 luminal A and luminal B tumors as defined by PAM50
- The best Ki67 index cut point to distinguish luminal B from luminal A tumors was 13.25%
  - Sensitivity 72% (95% CI = 59% to 82%)
  - Specificity 77% (95% CI = 67% to 85%)

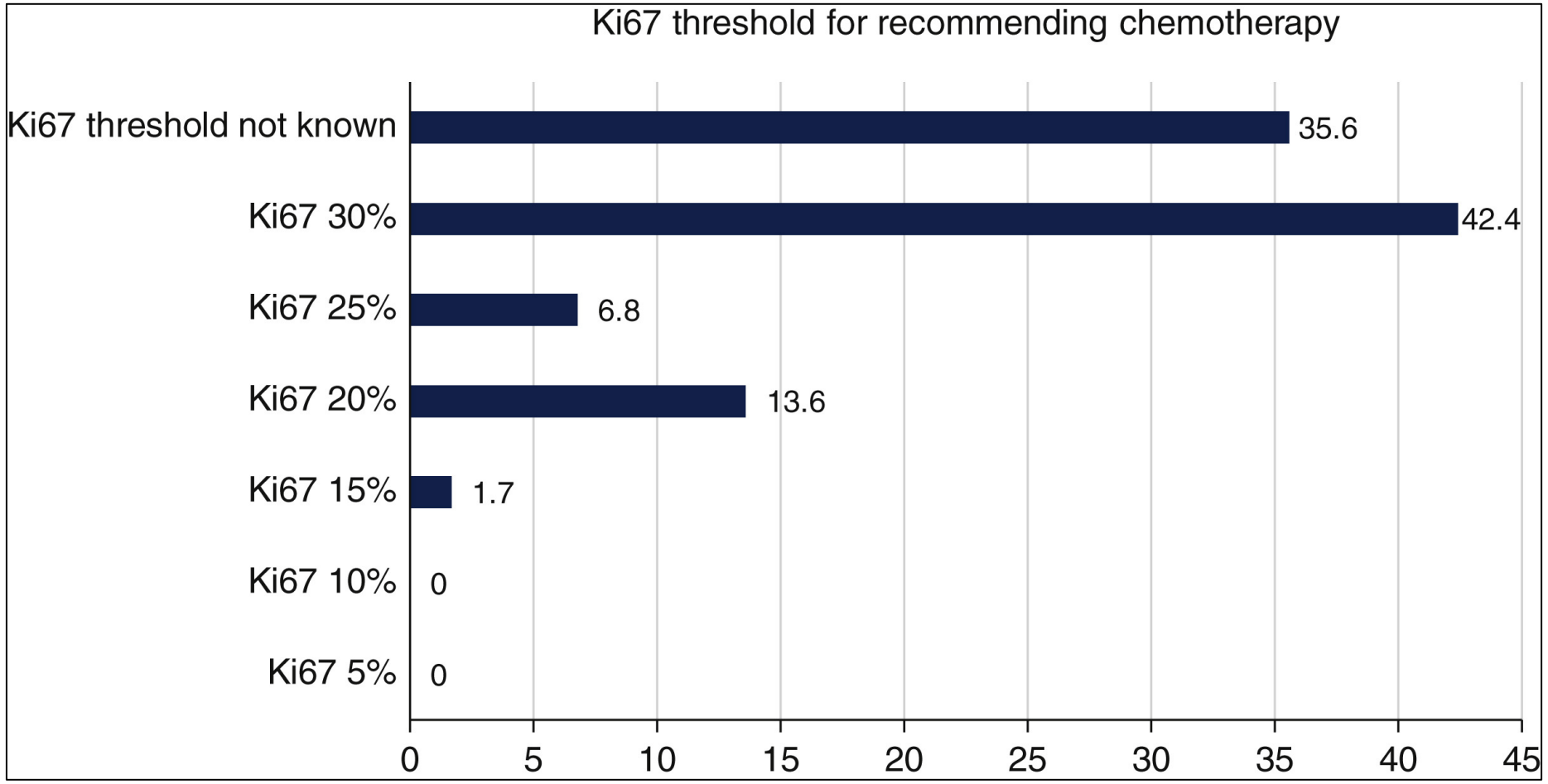
Cheang MCU et al *J Natl Cancer Inst*. 2009; 101:736-750

Immunohistochemistry surrogate to distinguish between luminal A and luminal B breast cancer subtypes

	St Gallen Consensus 2011	St Gallen Consensus 2013
Luminal A-like	ER and/or PR-positive, HER2-negative **Ki-67 low (<14%)**	ER and PR-positive, HER2-negative **Ki-67 low (<20%)** Recurrence risk low based on multigene testing
Luminal B-like (HER2-negative)	ER and/or PR-positive, HER2-negative **Ki-67 high**	ER-positive, HER2-negative And at least one of the following: **Ki-67 high** PR-negative or low Recurrence risk high based on multigene testing
Luminal B-like (HER2-positive)	ER and/or PR-positive, HER2-potitive Any Ki67	ER-positive, HER2-positive Any Ki-67 Any PR
HER2-like	HER2-positive, ER/PR-negative	HER2-positive, ER/PR-negative
Basal-like	ER/PR-negative, HER2-negative	ER/PR-negative, HER2-negative

Goldhirsch A. et al *Ann Oncol*. 2011; 22:1736-1747. Goldhirsch A et al. *Ann Oncol*. 2013; 24:2206-2223

St Gallen International Consensus 2021



Burstein HJ et al. *Ann Oncol*. 2021; 32:1216-1235

International Ki67 in Breast Cancer Working Group (IKWG)

OXFORD

JNCI J Natl Cancer Inst (2021) 113(7): djaa201

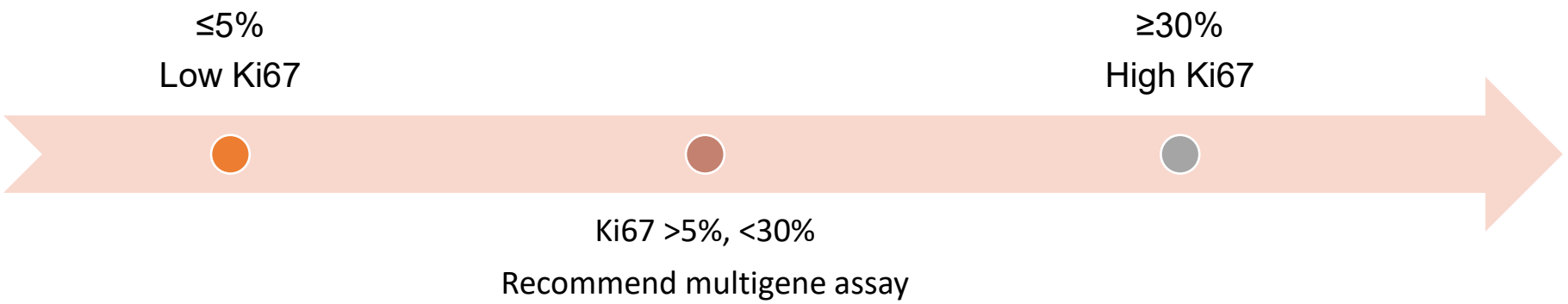
doi: 10.1093/jnci/djaa201
First published online December 28, 2020
Commentary

Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group

Torsten O. Nielsen, MD, PhD, FRCPC,[1,*] Samuel C. Y. Leung, MSc,[1] David L. Rimm, MD, PhD,[2] Andrew Dodson, MPhil, FIBMS, CSci,[3] Balazs Acs, MD, PhD,[4,5] Sunil Badve, MBBS, MD, FRCPath,[6] Carsten Denkert, MD,[7] Matthew J. Ellis, MB, BChir, BSc, PhD, FRCP,[8] Susan Fineberg, MD,[9] Margaret Flowers, PhD,[10] Hans H. Kreipe, MD,[11] Anne-Vibeke Laenkholm, MD,[12] Hongchao Pan, PhD,[13] Frédérique M. Penault-Llorca, MD, PhD,[14] Mei-Yin Polley, PhD,[15] Roberto Salgado, MD, PhD,[16,17] Ian E. Smith, MD, FRCP, FRCPE,[18] Tomoharu Sugie, MD, PhD,[19] John M. S. Bartlett, BSc, PhD, FRCPath,[20,21] Lisa M. McShane, PhD,[22] Mitch Dowsett, BSc, PhD[23,] Daniel F. Hayes MD[24,]

IKWG updated recommendations

- Pre-analytical standardization
  - Tissue collection, fixation and processing follow the ASCO/CAP guidelines for ER, PR, HER2
- Establish standardized scoring method
- Clinical utility: In T1-2, N0-1 ER-positive, HER2-negative breast cancer, Ki67 5% or less, or 30% or more, can be used to estimate prognosis



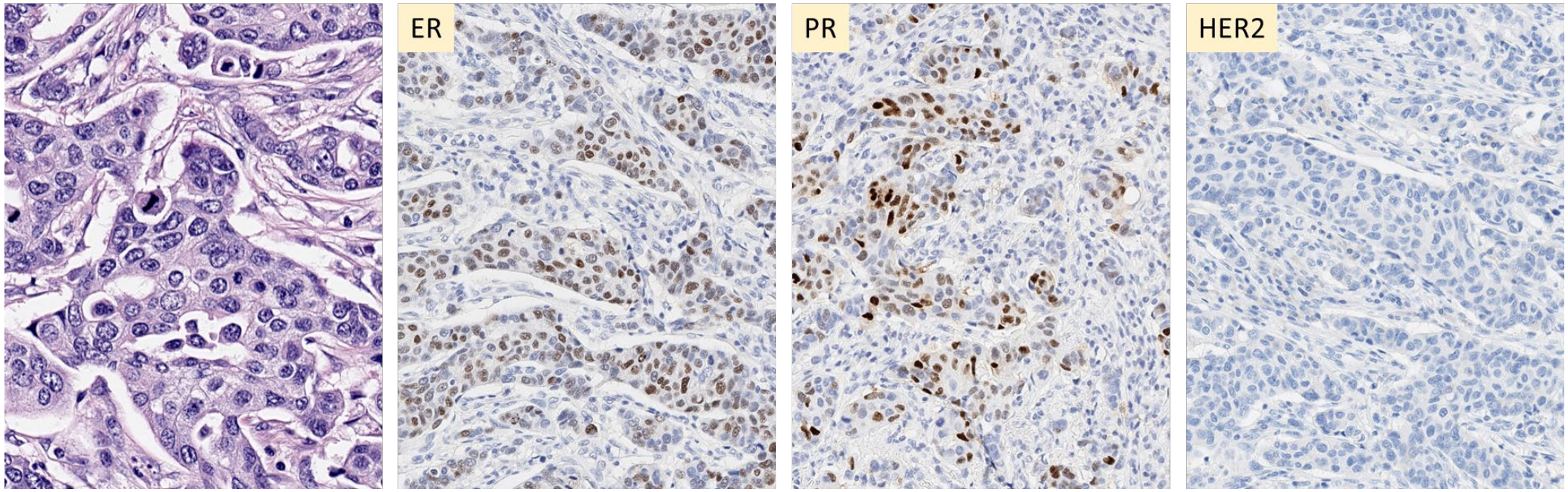
Nielsen TO et al. *J Natl Cancer Inst*. 2021; 113:808-819

Risk stratification by Ki67 IKWG and 21-gene RS: low concordance

Ki67 IHC	IKWG	Oncotype Dx RS ≤25	Oncotype Dx RS >25
MIB	Ki67 ≤5% (n=8)	5	3
	Ki67 ≥30% (n=10)	6	4
30-9	Ki67 ≤5% (n=4)	3	1
	Ki67 ≥30% (n=16)	12	4

Krystel-Whittemore M et al. *USCAP 2022 Annual Meeting*

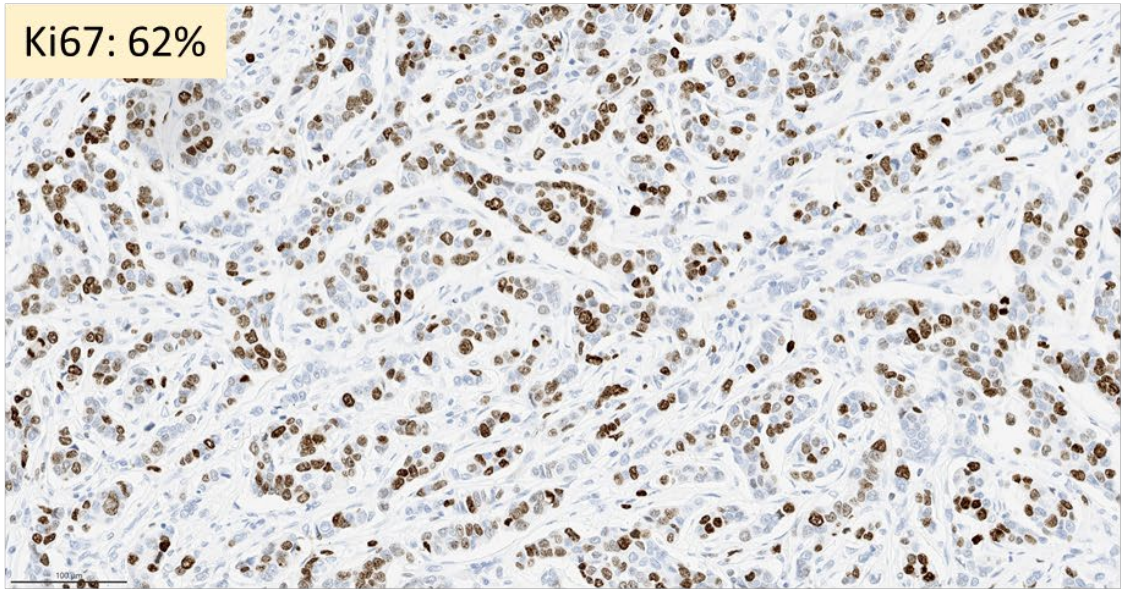
Case example



38 yo woman, invasive ductal carcinoma, NOS type, grade III, pT1c N0

ER 60%, PR 40%, HER2 0

Ki67 vs RS: concordance



Recurrence Score® (RS) Result	Distant Recurrence Risk at 9 Years	Group Average Absolute Chemotherapy (CT) Benefit*
40	With TAM Alone 28% 95% CI (21%, 36%) NSABP B-14	RS 26-100 All Ages >15% 95% CI (9%, 37%) NSABP B-20

Decision on individual treatment especially around the RS 25 cutoff may consider other clinical factors.

TAM = Tamoxifen
CI = Confidence Intervals

*For estimated CT benefit for individual RS results, see page 2.

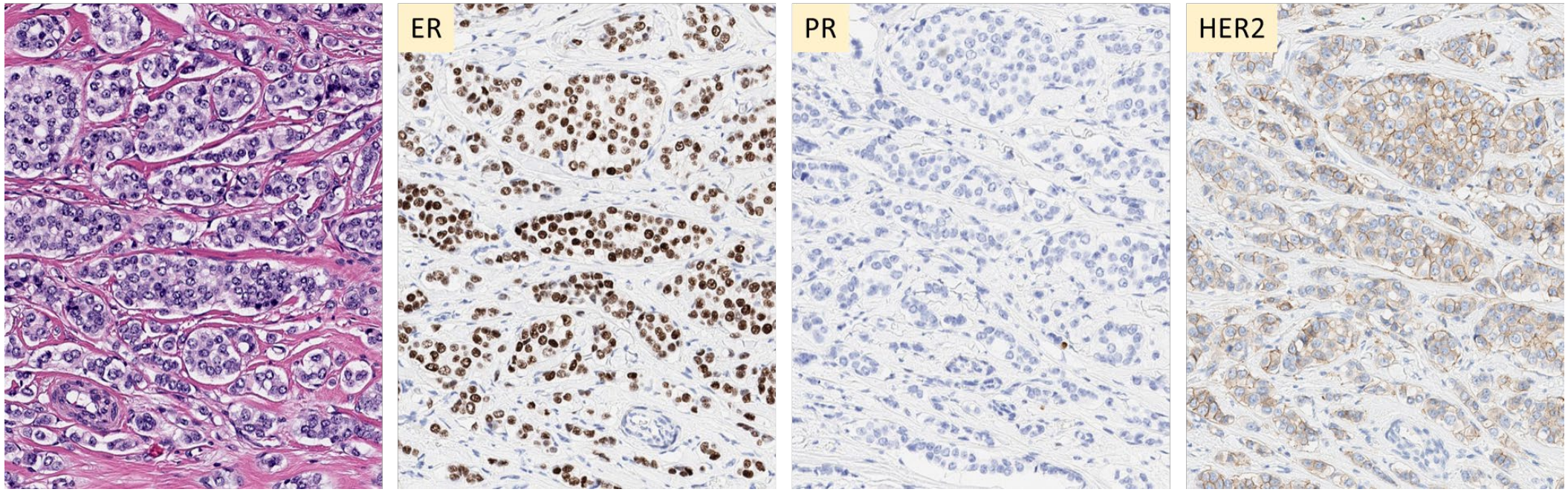
Exploratory Subgroup Analysis for TAILORx and NSABP B-20:
Absolute CT Benefit for Distant Recurrence by Age and RS Result

Age	RS 0-10	RS 11-15	RS 16-20	RS 21-25	RS 26-100
>50 years	No CT Benefit (<1%)				>15% CT Benefit
≤50 years	No CT Benefit (<1%)		~1.6% CT Benefit	~6.5% CT Benefit	>15% CT Benefit

Quantitative Single-Gene Scores

7.5 ER Positive	5.8 PR Positive	8.4 HER2 Negative
<3.7 6.5 ≥12.5	<3.2 5.5 ≥10.0	<7.6 10.7 11.5 ≥13.0

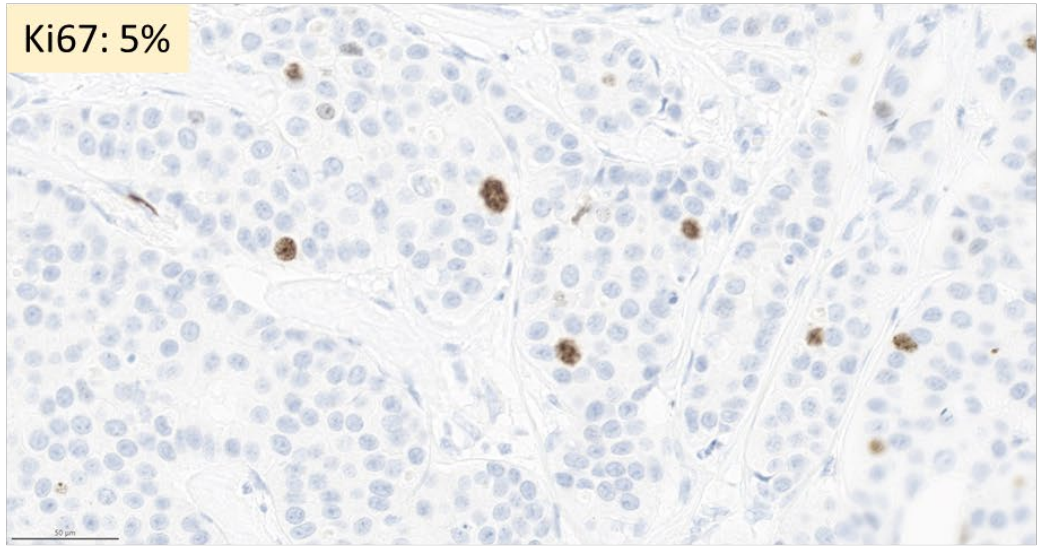
Case example

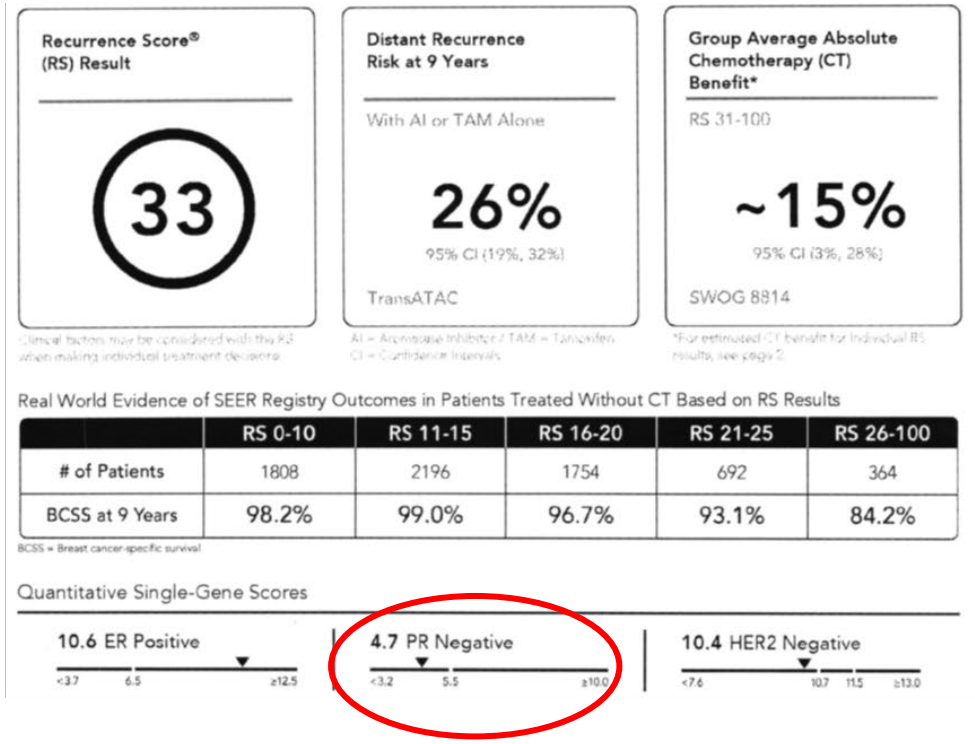


54 yo woman, invasive ductal carcinoma, NOS type, grade II, pT2 N1mi

ER 50%, PR 0, HER2 IHC 2+, FISH not amplified

Ki67 vs RS: discordant

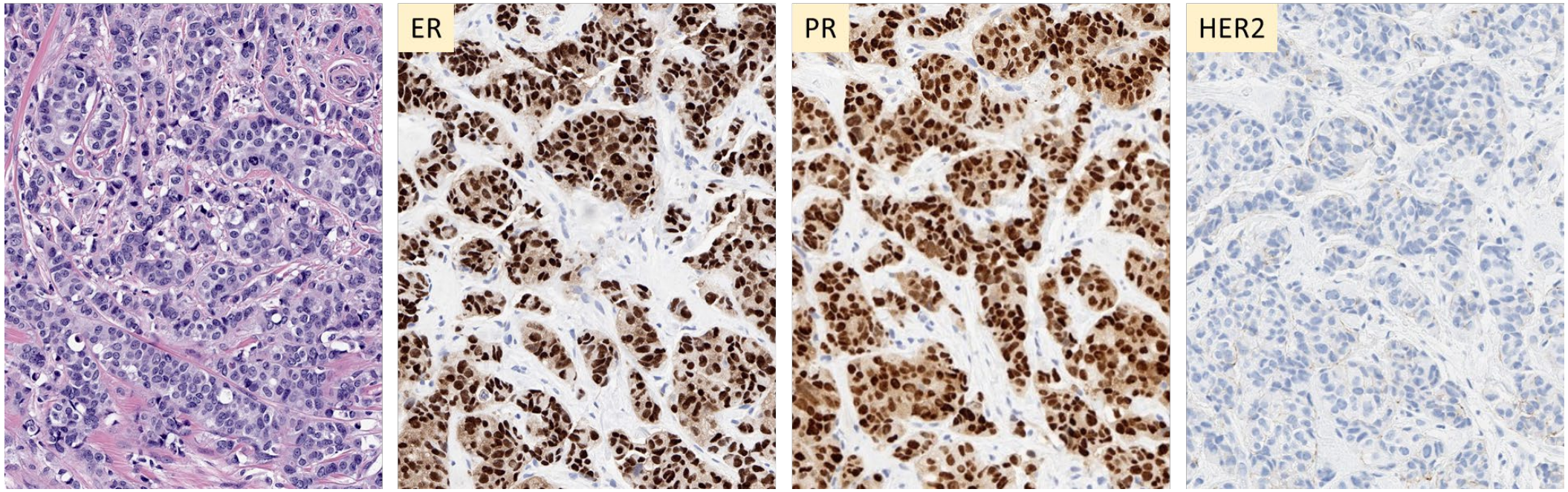




Low Ki67: 5%

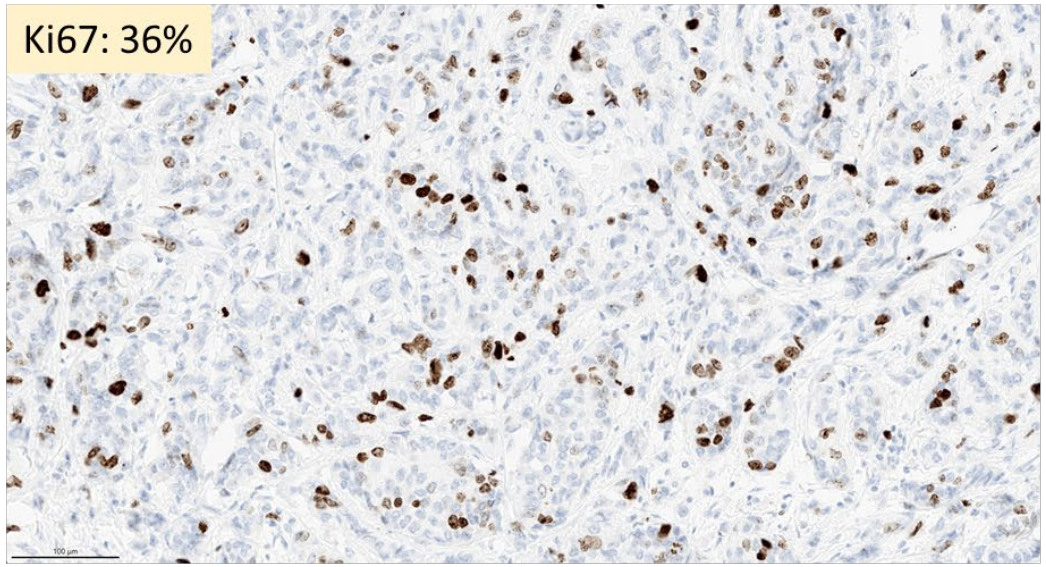
High risk recurrence score, RS=33

Case example



69 yo woman, invasive ductal carcinoma, NOS type, grade II, pT2N1mi
ER 99%, PR 99%, HER2 0

Ki67 vs RS: discordant



Recurrence Score® (RS) Result	Distant Recurrence Risk at 9 Years	Group Average Absolute Chemotherapy (CT) Benefit*
10	With AI or TAM Alone 12% 95% CI (8%, 16%) TransATAC	RS 0-17 No Apparent Benefit SWOG 8814

Real World Evidence of SEER Registry Outcomes in Patients Treated Without CT Based on RS Results

	RS 0-10	RS 11-15	RS 16-20	RS 21-25	RS 26-100
# of Patients	1808	2196	1754	692	364
BCSS at 9 Years	98.2%	99.0%	96.7%	93.1%	84.2%

BCSS = Breast cancer-specific survival

Quantitative Single-Gene Scores[1]

11.3 ER Positive

9.3 PR Positive

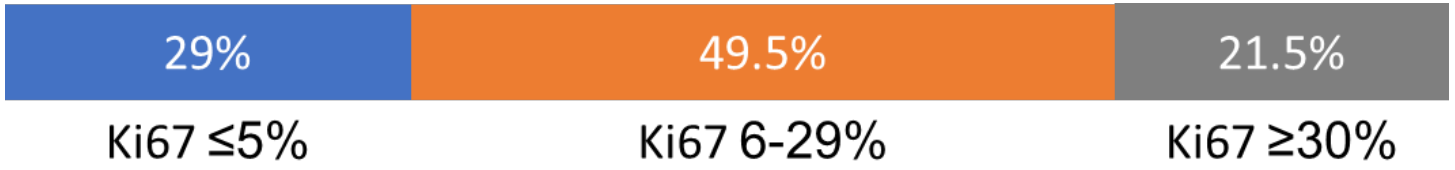
8.4 HER2 Negative

High Ki67: 36%

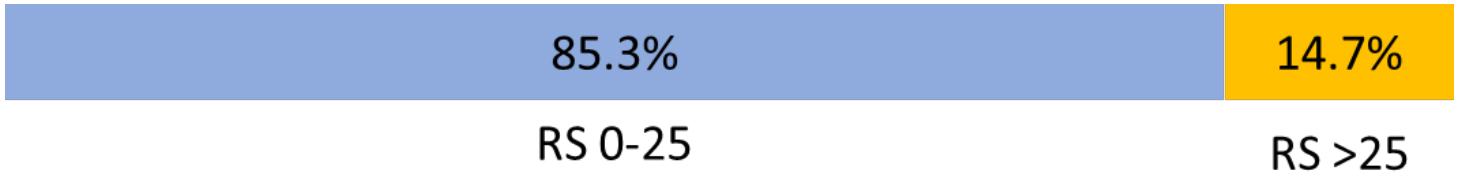
Low risk recurrence score RS=10

Correlation of the Ki67 IKWG risk categories with the Oncotype Dx recurrence score in early breast cancer

- A retrospective review of 525 HR-positive, HER2-negative, N0-1 early breast cancer
- Ki67



- 21-gene recurrence score



Patel R et al *Cancer* 2022; 128:3602-3609

Correlation between Ki67 and RS

	RS>25
Ki67 low (0-5%)	6.6%
Ki67 intermediate (6-29%)	11%
Ki67 high (≥30%)	34.5%

Patel R et al *Cancer* 2022; 128:3602-3609

IKWG Ki67 vs 21-gene recurrence score

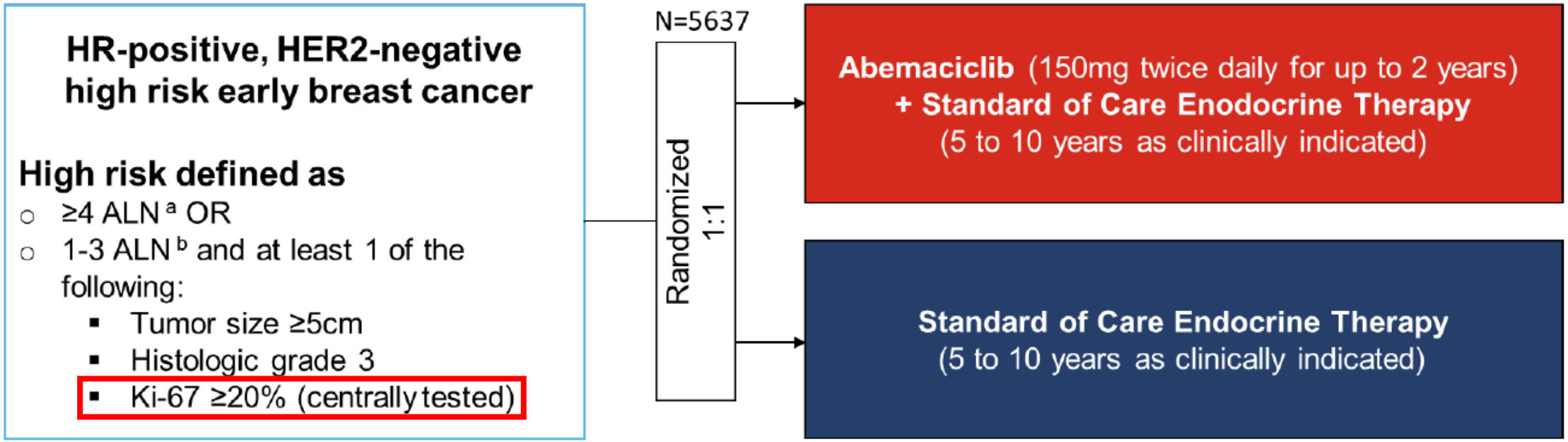
IKWG Ki67 does not significantly correlate with 21-gene recurrence score in retrospective studies

Outcome data?

Ki67 companion diagnostic assay for CDK4/6 inhibitor

MonarchE trial

Figure S1. Study diagram



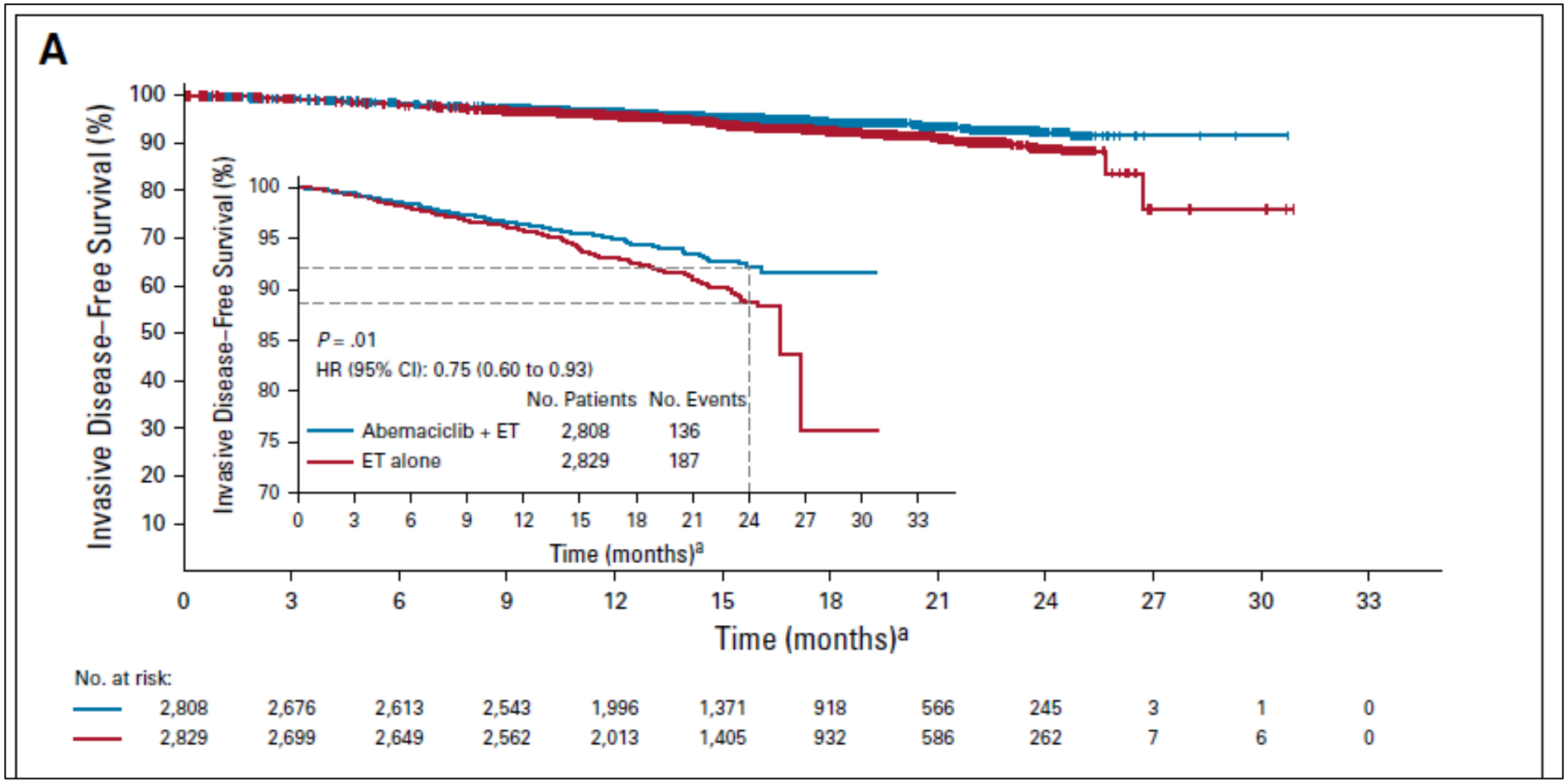
Additional criteria:
- Women or men
- Pre-/ postmenopausal
- With or without prior adjuvant/neoadjuvant chemotherapy
- No distant metastases

Stratification criteria:
- Menopausal Status
- Prior chemotherapy
- Region

Endocrine Therapy of investigator's discretion

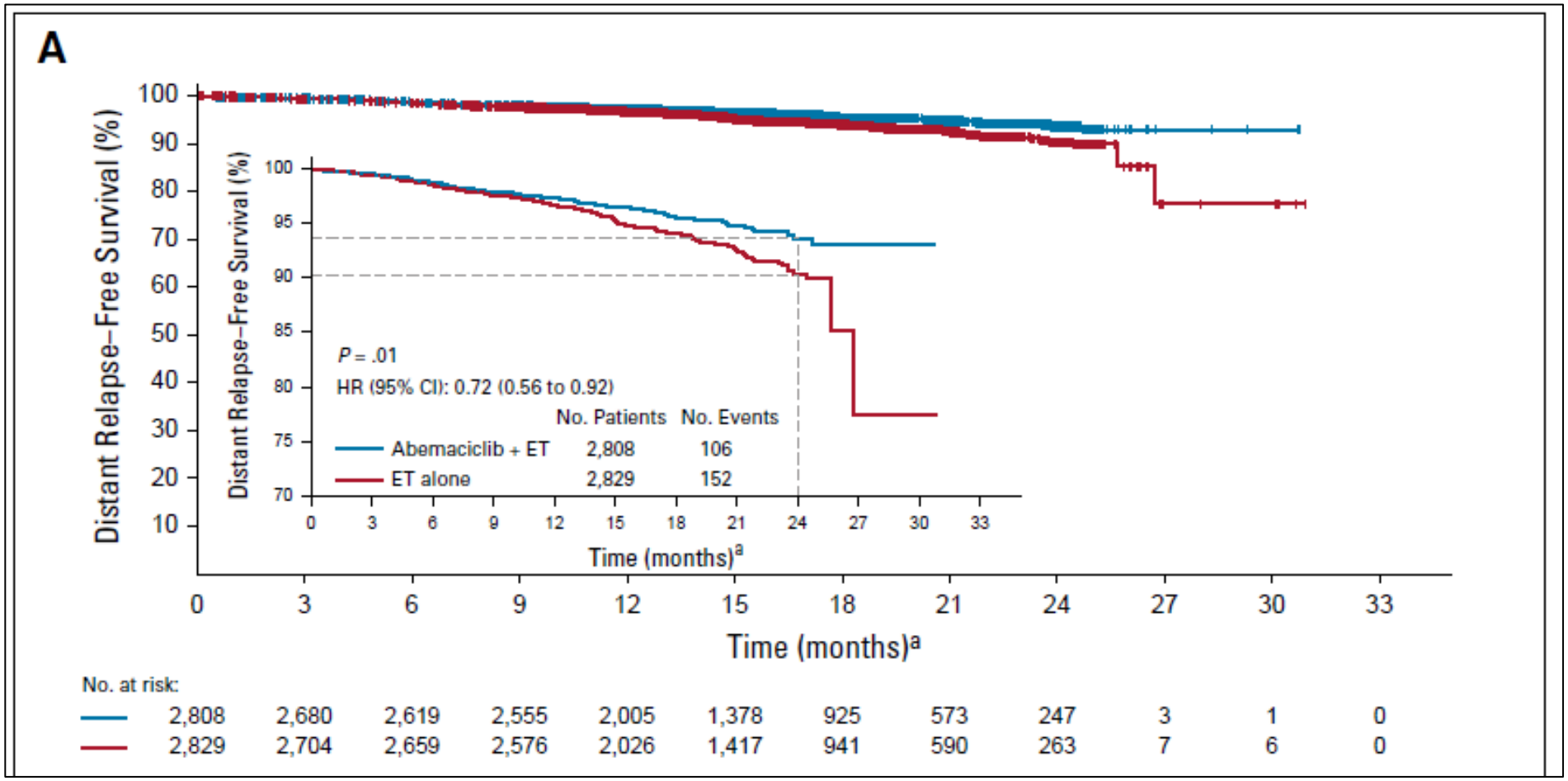
Johnston SRD et al. *J Clin Oncol.* 2020; 38:3987-3998

Abemaciclib plus ET demonstrated a statistically significant improvement in IDFS versus ET alone (*P* = .01; HR, 0.75; 95% CI, 0.60 to 0.93)



Johnston SRD et al. *J Clin Oncol.* 2020; 38:3987-3998

The addition of abemaciclib to ET also resulted in an improvement in DRFS compared with ET alone (nominal *P* = .01; HR, 0.72; 95% CI, 0.56 to 0.92)



Johnston SRD et al. *J Clin Oncol.* 2020; 38:3987-3998

FDA approves abemaciclib with endocrine therapy for early breast cancer

On October 12,2021, the Food and Drug Administration approved abemaciclib (Verzenio,Eli Lilly and Company)with endocrine therapy (tamoxifen or an aromatase inhibitor) for adjuvant treatment of adult patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, node-positive, early breast cancer at high risk of recurrence and a Ki-67 score ≥20%, as determined by an FDA approved test. This is the first CDK 4/6 inhibitor approved for adjuvant treatment of breast cancer.

FDA also approved the Ki-67 IHC MIB-1 pharmDx (Dako Omnis) assay, submitted by Agilent, Inc., as a companion diagnostic for selecting patients for this indication.

Updated efficacy and Ki-67 analysis from the monarchE study

Ki-67 index was prognostic, but abemaciclib benefit was observed regardless of Ki-67 index

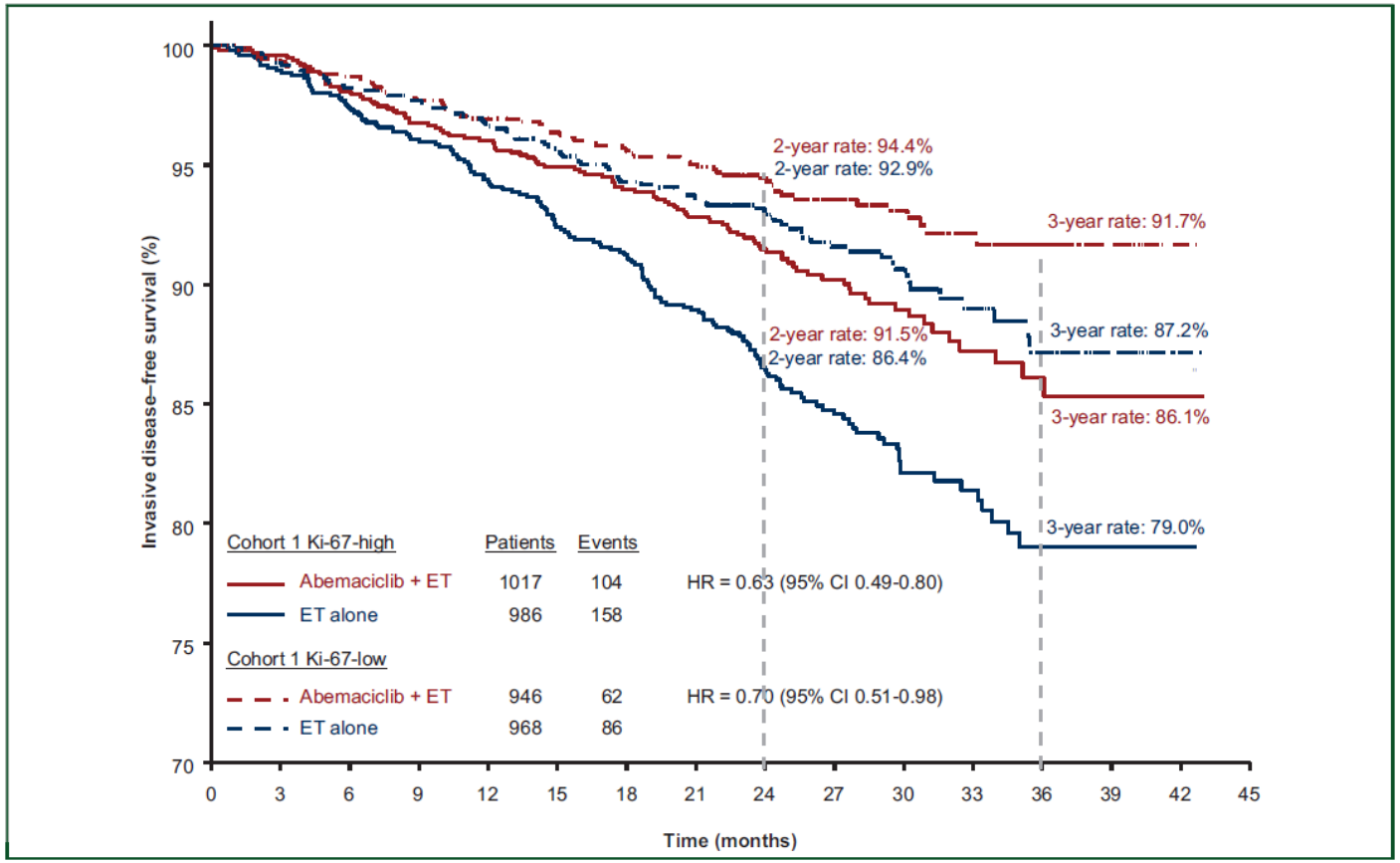


Figure 3. Kaplan-Meier curves of invasive disease-free survival in Cohort 1 Ki-67 high versus Ki-67 low at additional follow-up 1 (AFU1).
CI, confidence interval; ET, endocrine therapy; HR, hazard ratio.

Harbeck N et al *Ann Oncol*. 2021; 32:1571-1581

FDA expands early breast cancer indication for abemaciclib with endocrine therapy

On March 3, 2023, the Food and Drug Administration (FDA) approved abemaciclib (Verzenio, Eli Lilly and Company) with endocrine therapy (tamoxifen or an aromatase inhibitor) for the adjuvant treatment of adult patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, node-positive, early breast cancer at high risk of recurrence.

Patients defined as high risk included those having either ≥4 pALN (pathologic axillary lymph nodes) or 1-3 pALN and either tumor grade 3 or a tumor size ≥50 mm.

Abemaciclib was previously approved for the above high-risk population with the additional requirement of having a Ki-67 score ≥20%. Today's approval removes the Ki-67 testing requirement.

Multigene assays

Multigene assays for early breast cancer

21-gene recurrence score assay (Oncotype Dx™)

70-gene signature (MammaPrint™)

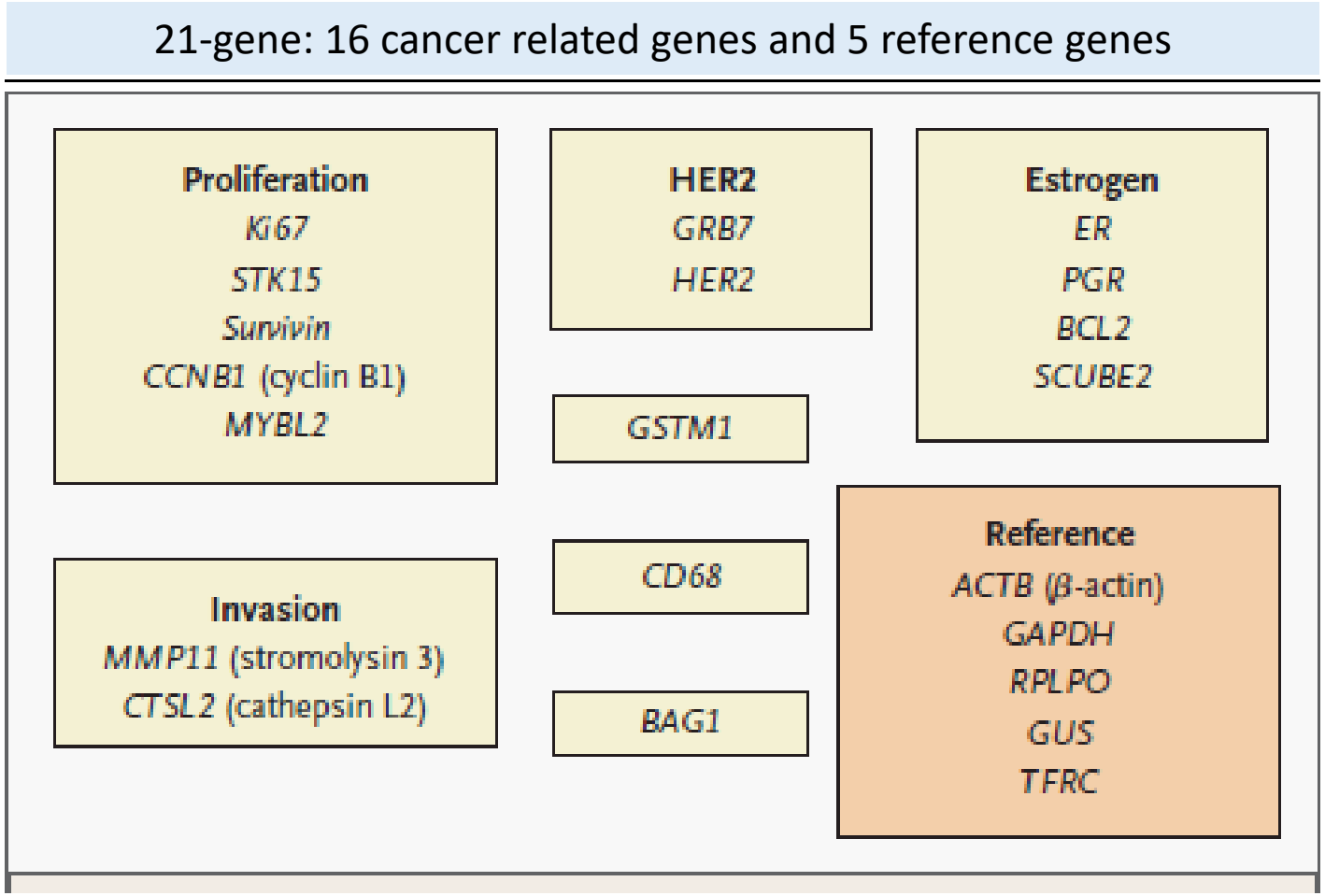
12-gene risk score (EndoPredict™)

PAM50 (Prosigna™)

Breast Cancer Index (BCI)

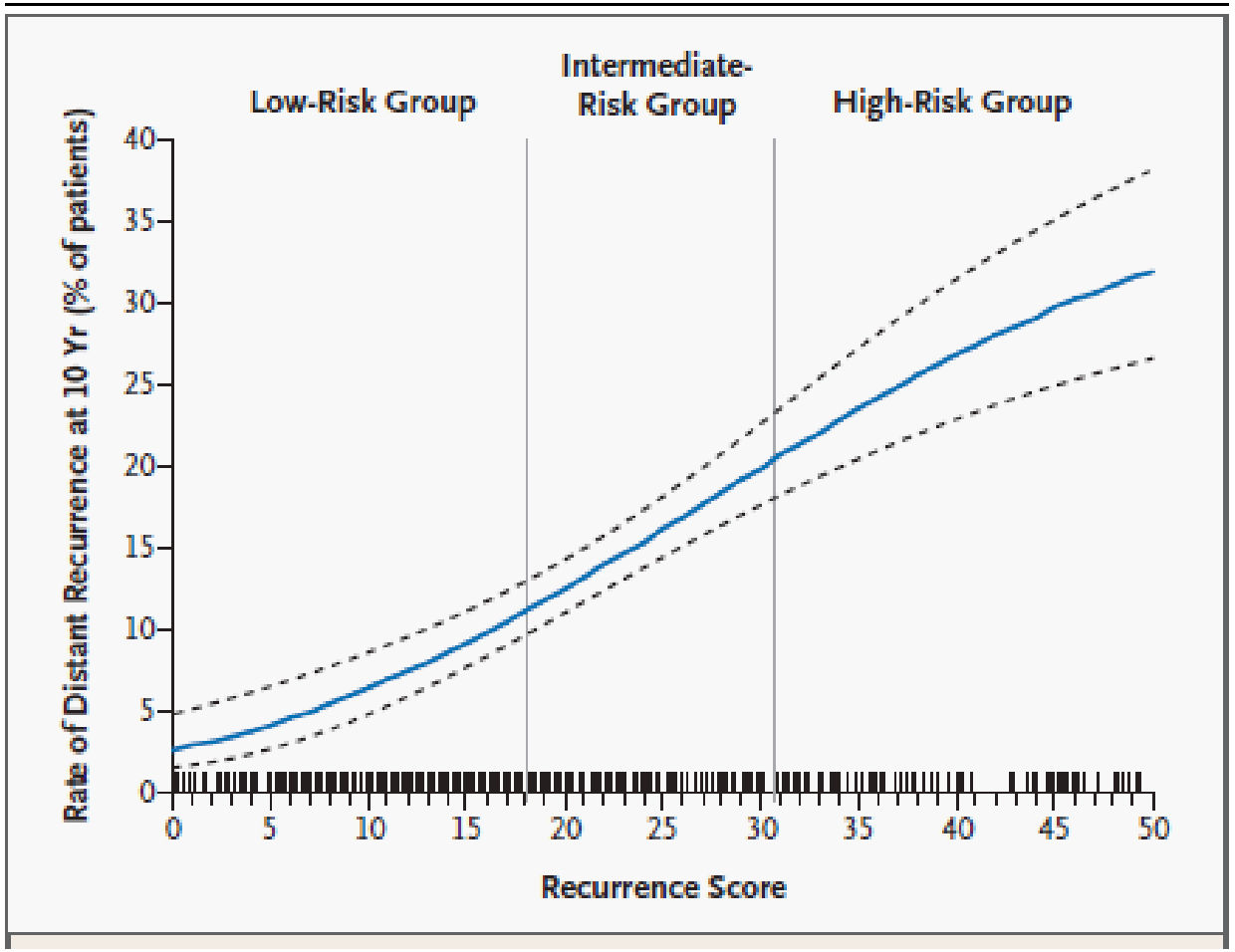
21-gene recurrence score assay (RT-PCR assay)

- RS_U =
  - + 0.47 × *GRB7* group score
  - – 0.34 × *ER* group score
  - **+ 1.04 × proliferation group score**
  - + 0.10 × invasion group score
  - + 0.05 × *CD68*
  - – 0.08 × *GSTM1*
  - – 0.07 × *BAG1*.



Paik S et al. *N Engl J Med*. 2004; 351:2817-2826

Rate of distant recurrence as a continuous function of the recurrence score



- The recurrence scores range from 0 to 100, with higher scores indicating a higher risk of distant recurrence

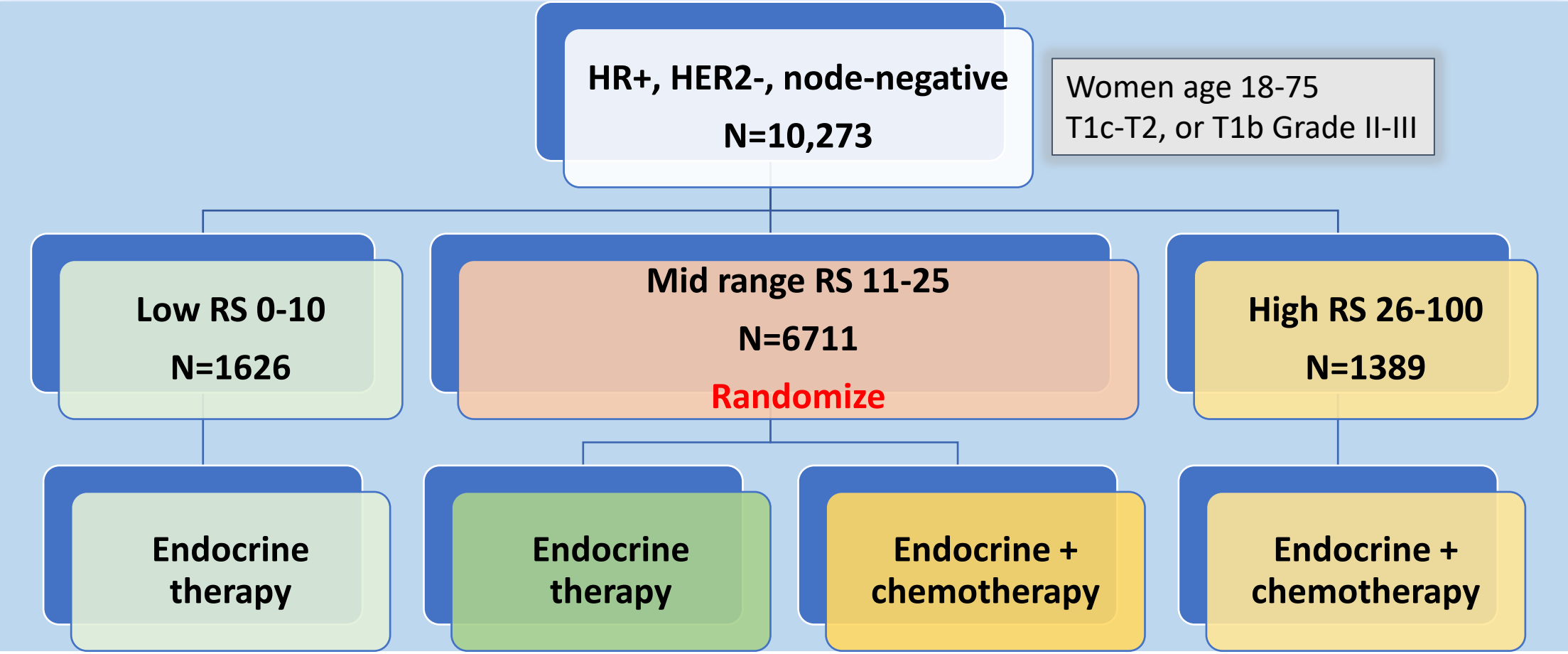
Paik S et al. *N Engl J Med*. 2004; 351:2817-2826

Prospective validation studies in patients with early-stage HR-positive HER2-negative breast cancer

Node-negative	Node-positive (1-3 positive nodes)
TAILORx trial	RxPONDER trial
10,273 patients	5083 patients
Recurrence score 0-25	Recurrence score 0-25

TAILORx trial design

(The Trial Assigning Individualized Options for Treatment)



Sparano JA. *Clin Breast Cancer*. 2006; 7:347-350

TAILORx
RS 0-10 (n=1626)

- Very low rates of recurrence at 5 years with endocrine therapy alone

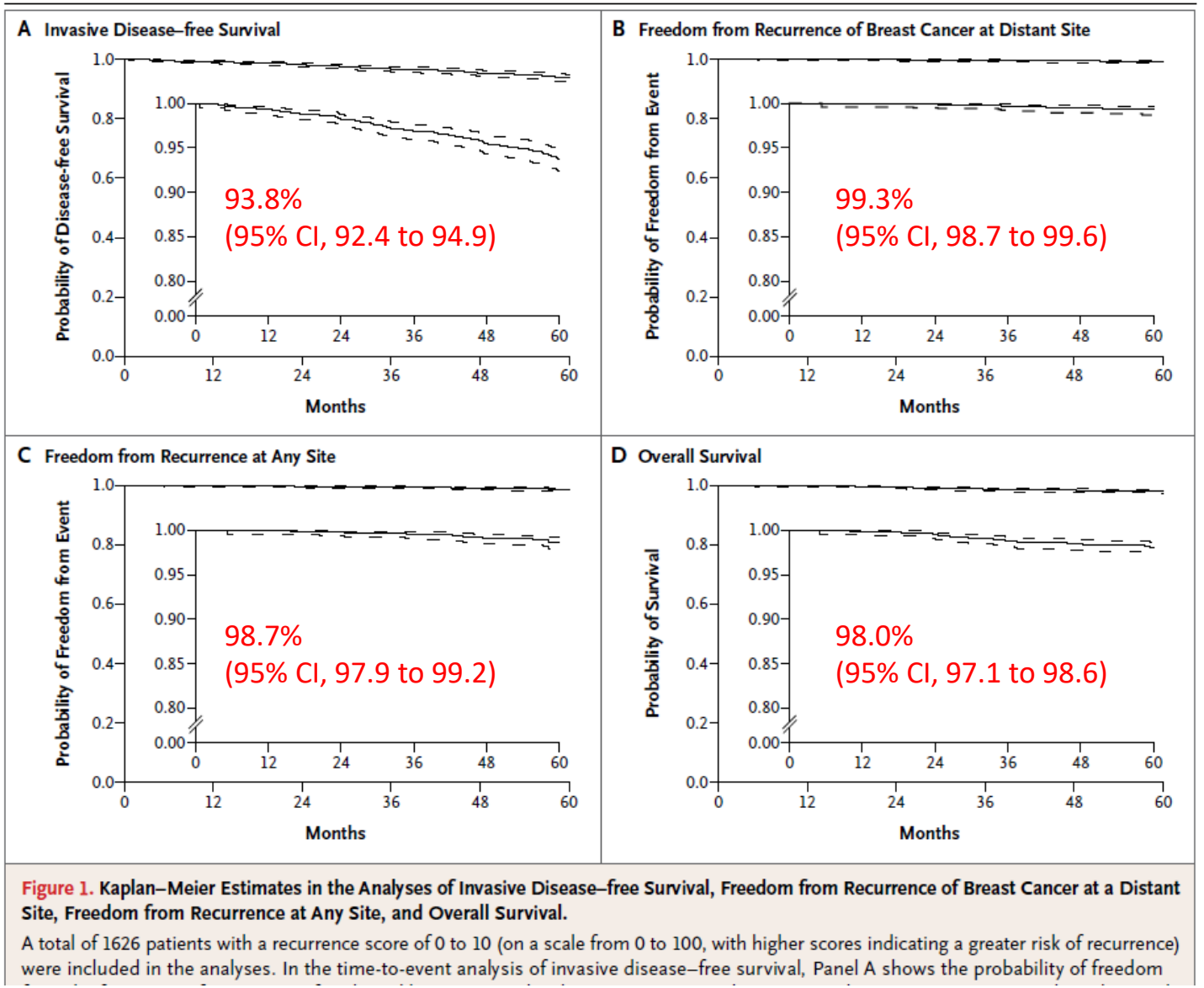


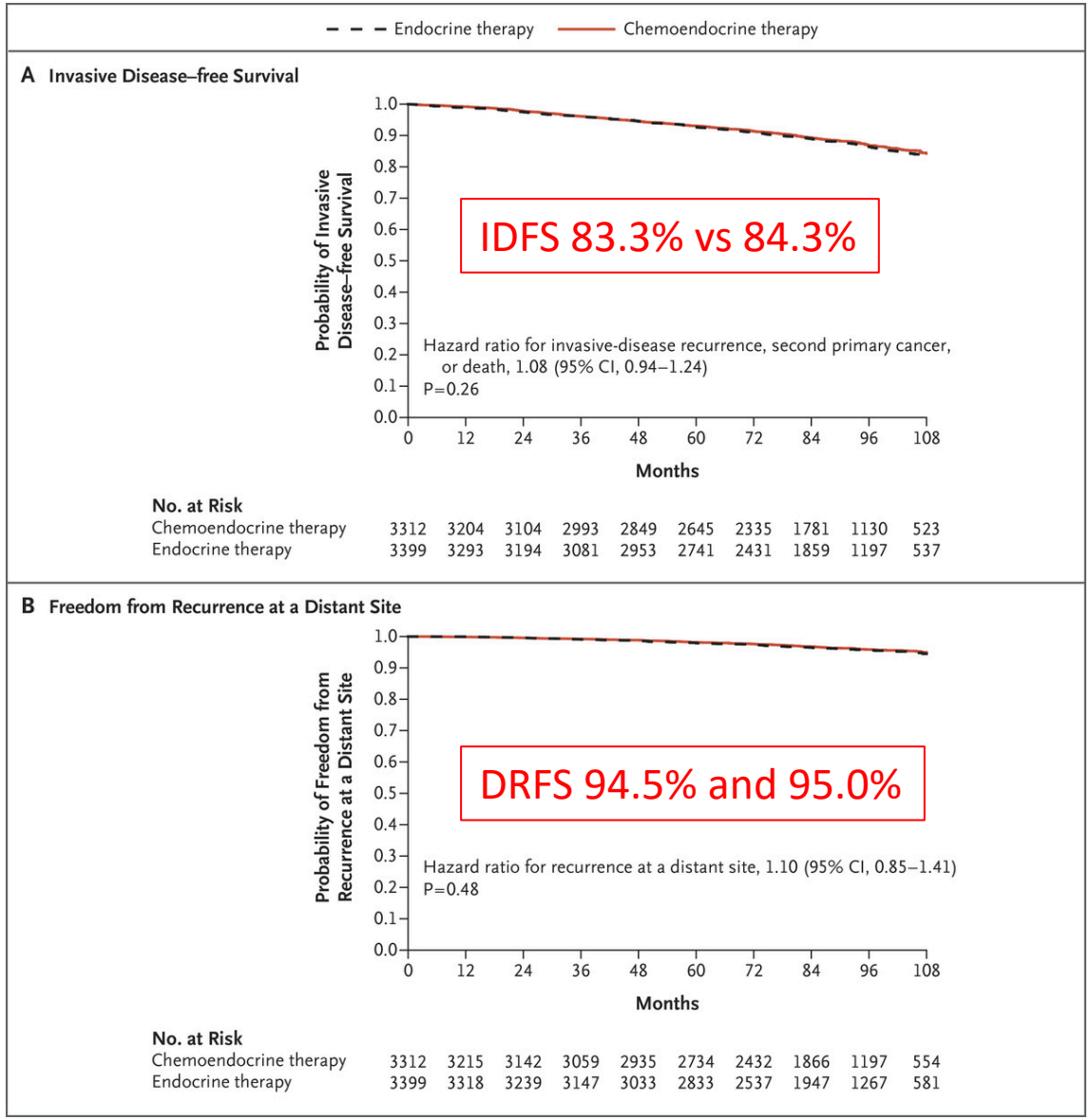
Figure 1. Kaplan–Meier Estimates in the Analyses of Invasive Disease–free Survival, Freedom from Recurrence of Breast Cancer at a Distant Site, Freedom from Recurrence at Any Site, and Overall Survival.

A total of 1626 patients with a recurrence score of 0 to 10 (on a scale from 0 to 100, with higher scores indicating a greater risk of recurrence) were included in the analyses. In the time-to-event analysis of invasive disease–free survival, Panel A shows the probability of freedom

Sparano JA et al. *N Engl J Med*. 2015; 373:2005-2014

TAILORx
RS 11-25 (N=6711)

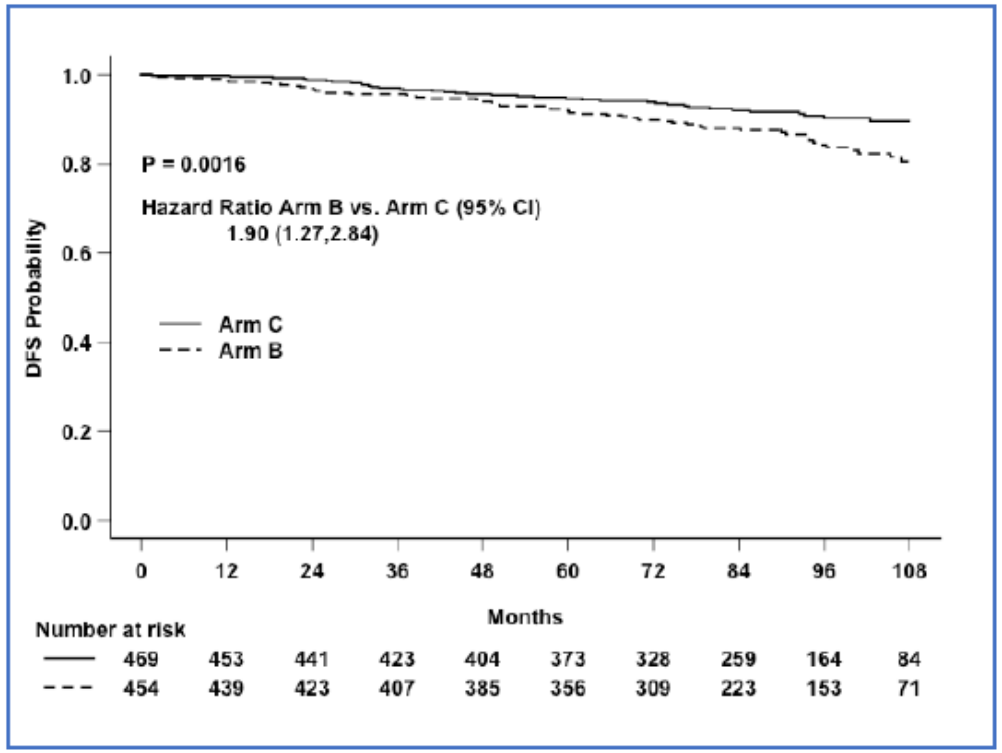
- Endocrine therapy was not inferior to chemo-endocrine therapy
- Adjuvant chemotherapy was not beneficial in these patients



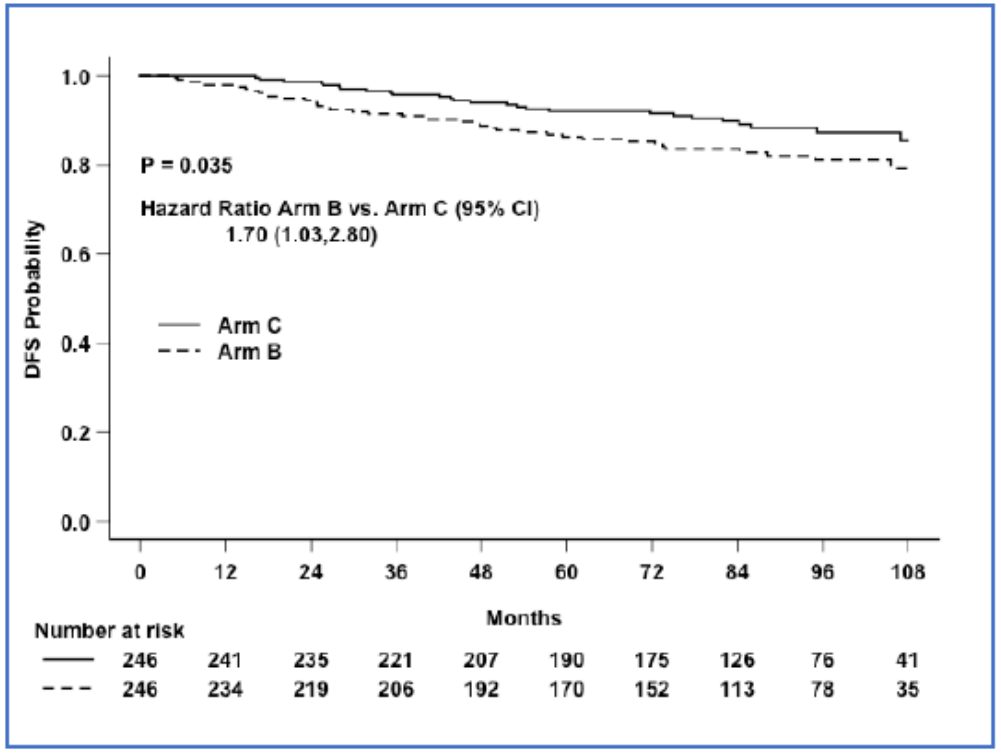
Sparano JA et al. *N Engl J Med*. 2018; 379:111-121

Some benefit of chemotherapy in young women (≤50 years) and RS 16-25

RS 16-20



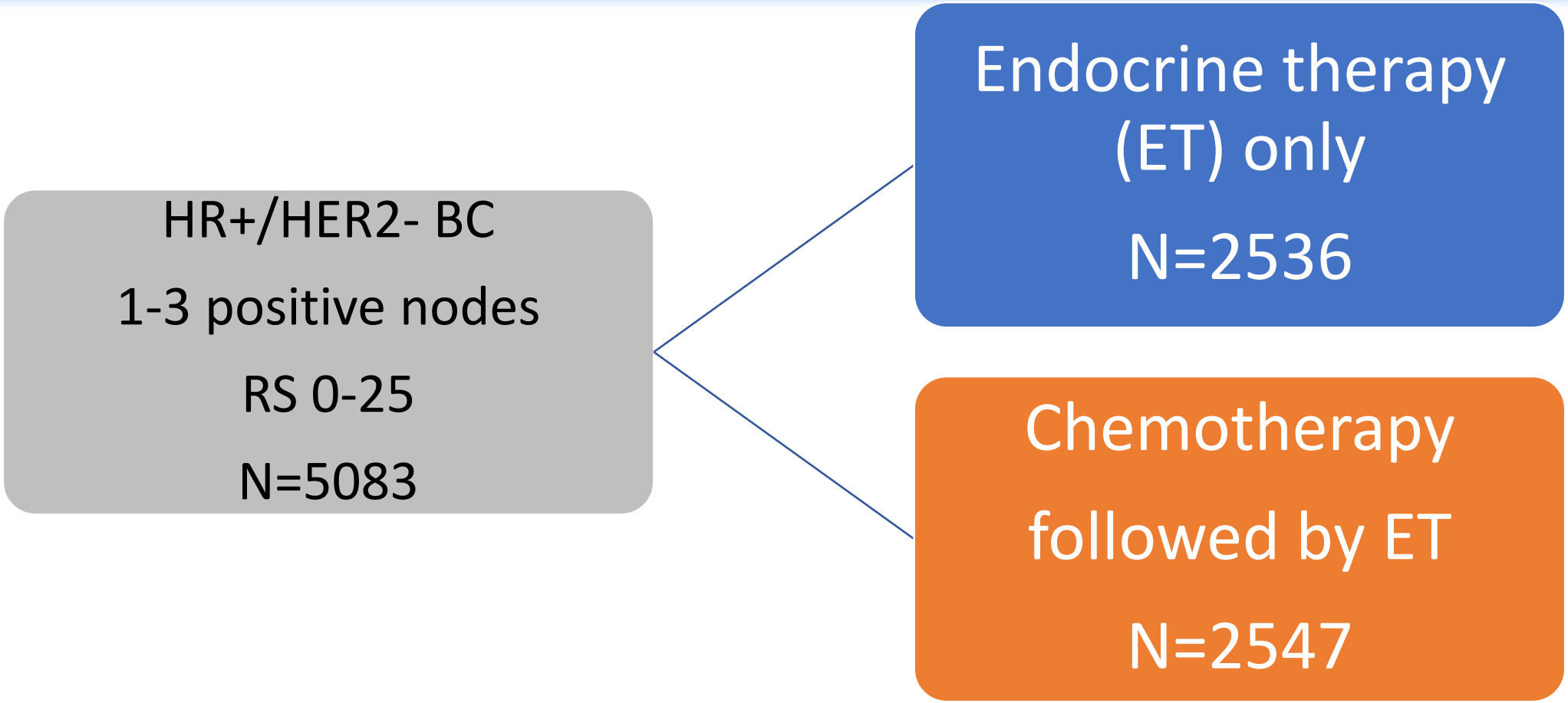
RS 21-25



Sparano JA et al. *N Engl J Med*. 2018; 379:111-121

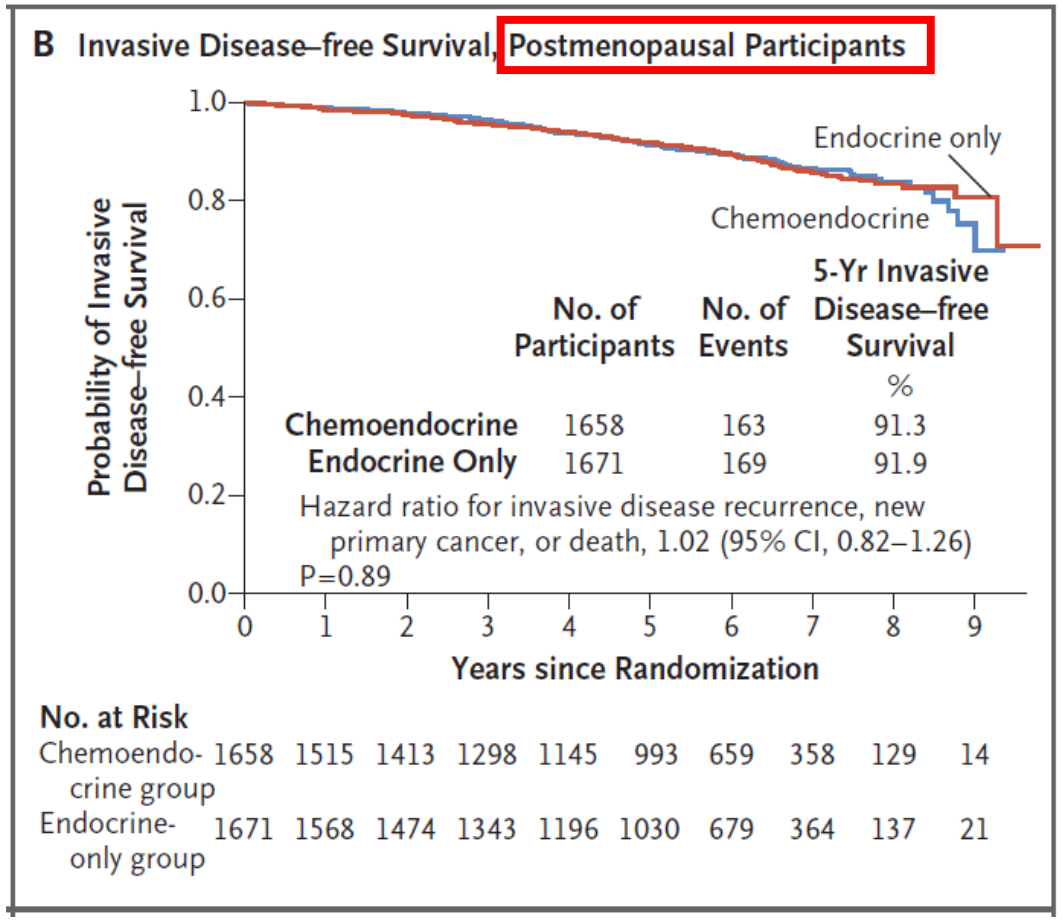
RxPONDER study design

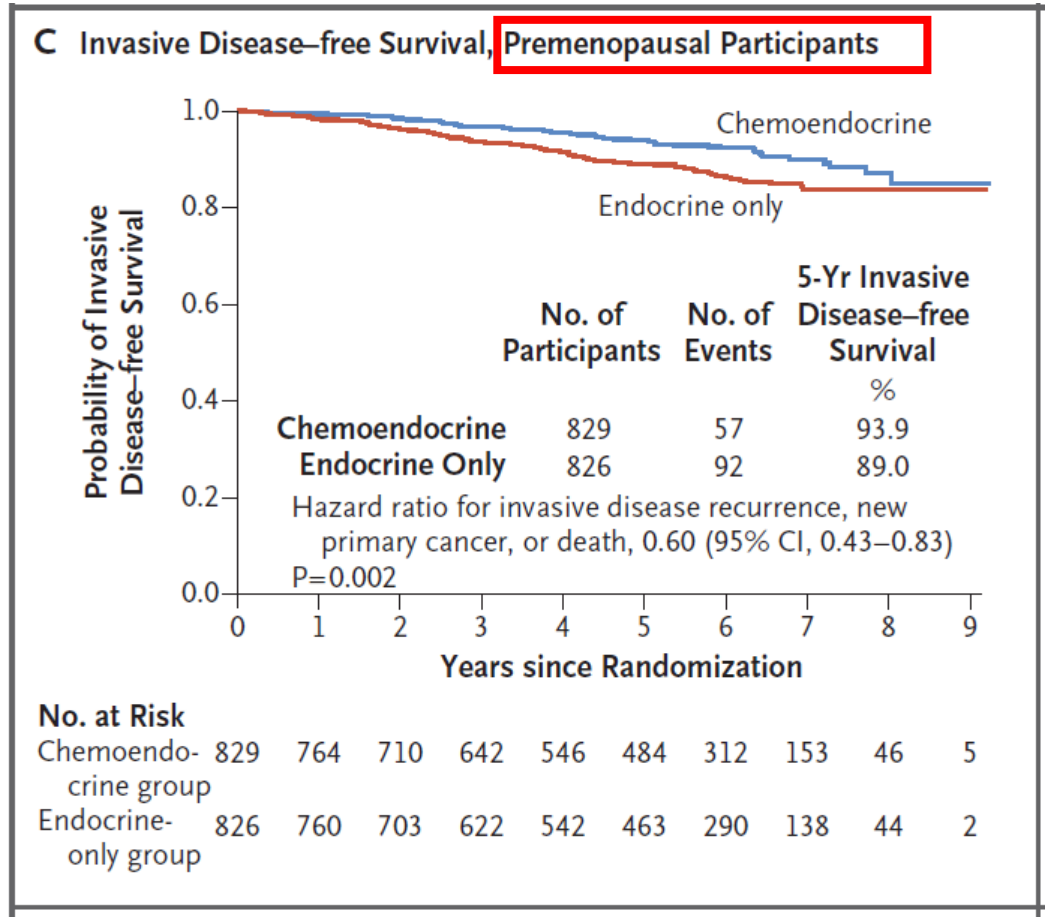
(A Clinical Trial RX for Positive Node, Endocrine Responsive Breast Cancer)



Postmenopausal women: No chemotherapy benefit

Premenopausal women: Significant benefit from chemotherapy even with low RS



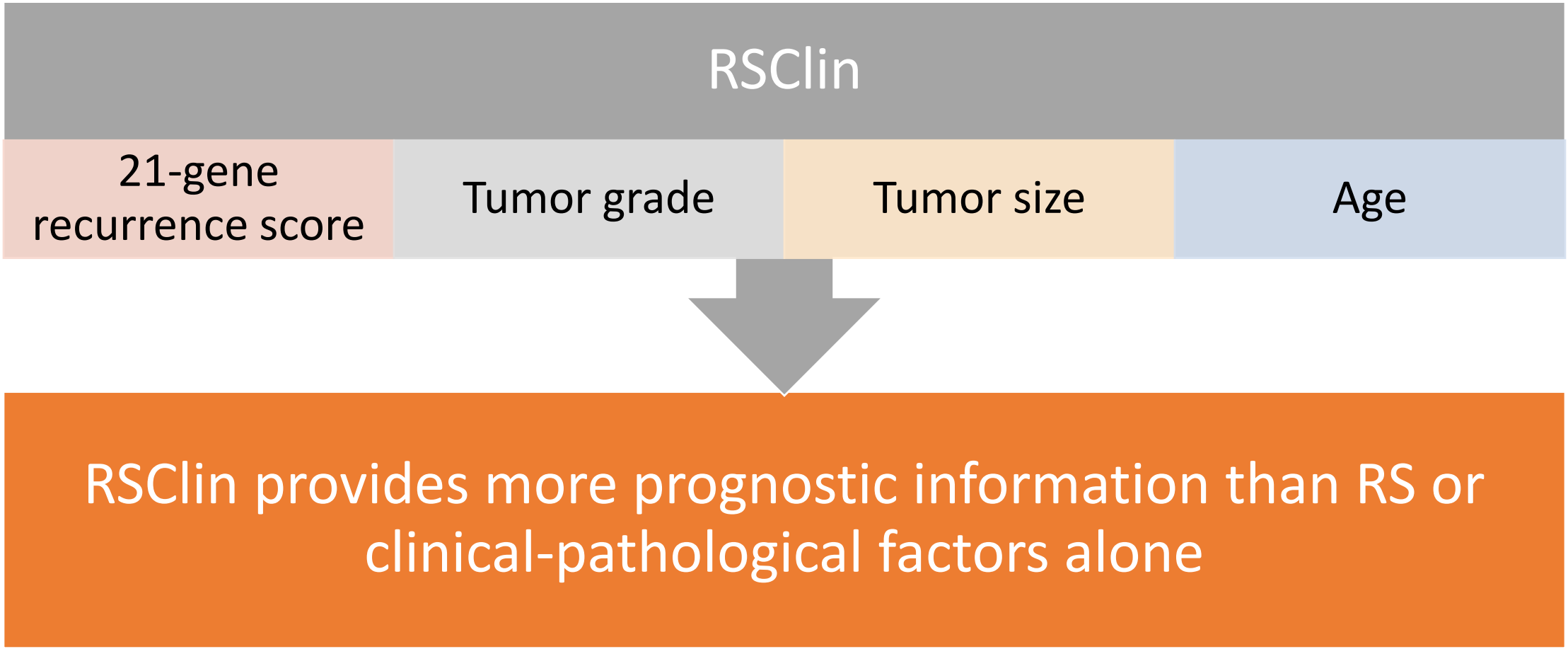


Kalinsky K et al. *N Engl J Med*. 2021; 385:2336-2347

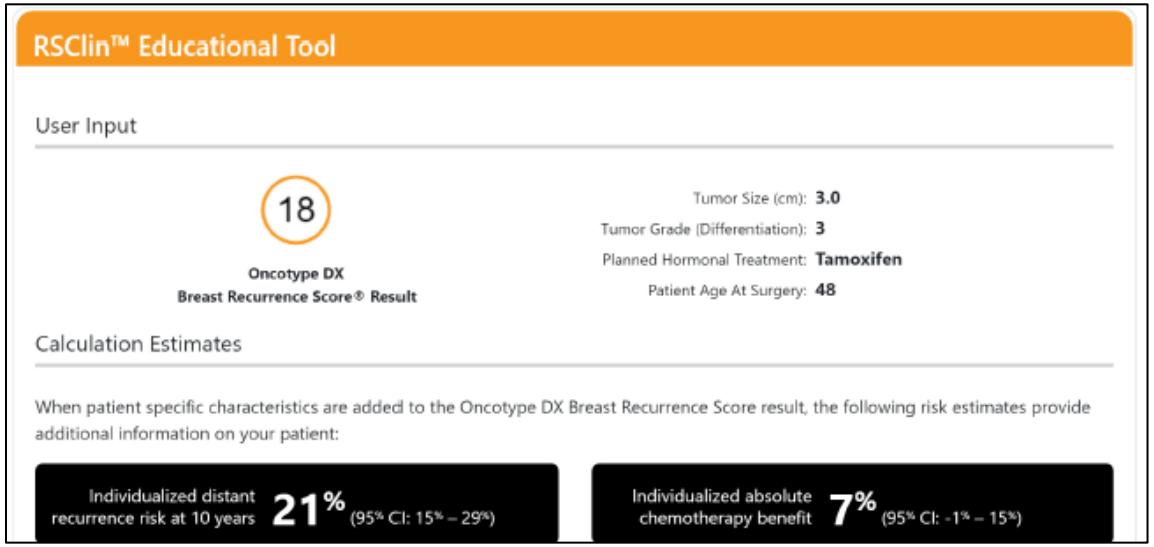
Chemotherapy benefit by age, nodal status, and RS in patients with early-stage ER-positive, HER2-negative breast cancer

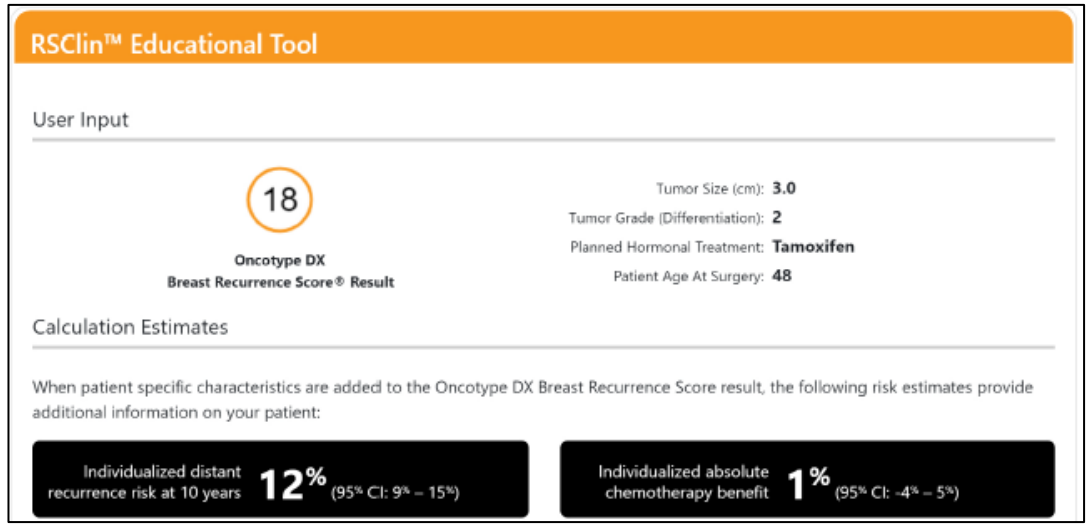
Age	Nodal status	RS 0-10	RS 11-15	RS 16-20	RS 21-25	RS ≥26
>50 years	Node-negative or 1-3 positive nodes	No chemotherapy benefit (<1%)				>15% benefit
≤50 years	Node-negative	No chemotherapy benefit (<1%)		~1.6% benefit	~6.5% benefit	>15% benefit
	Node-positive	RS does not apply				

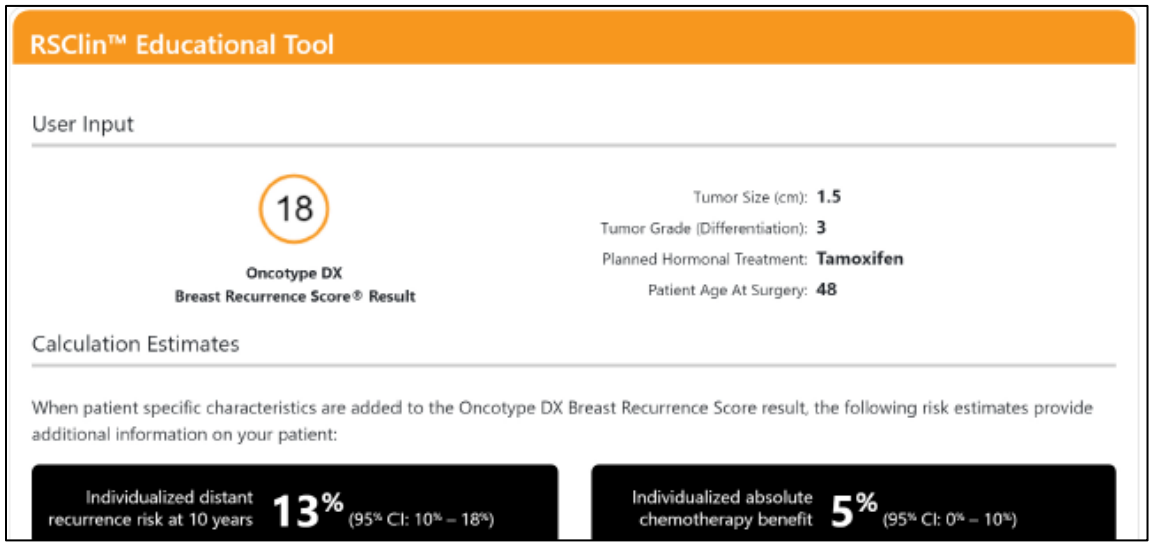
RSClin for HR+/HER2-, node-negative breast cancer

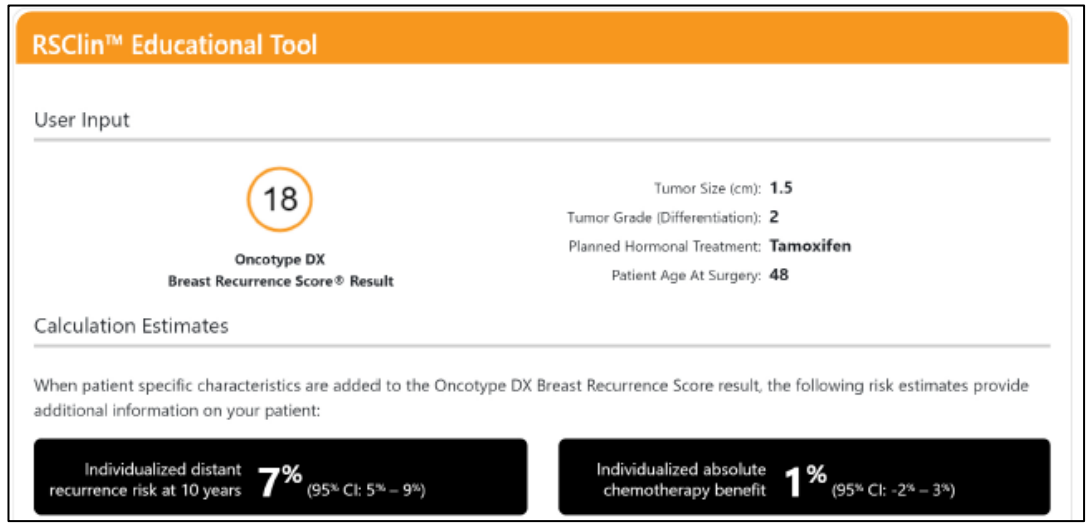


Sparano JA et al. *J Clin Oncol*. 2021; 39:557-564


RSClin™ Educational Tool
User Input
18
Oncotype DX
Breast Recurrence Score® Result
Tumor Size (cm): 3.0
Tumor Grade (Differentiation): 3
Planned Hormonal Treatment: Tamoxifen
Patient Age At Surgery: 48
Calculation Estimates
When patient specific characteristics are added to the Oncotype DX Breast Recurrence Score result, the following risk estimates provide additional information on your patient:
Individualized distant recurrence risk at 10 years 21% (95% CI: 15% – 29%)
Individualized absolute chemotherapy benefit 7% (95% CI: -1% – 15%)

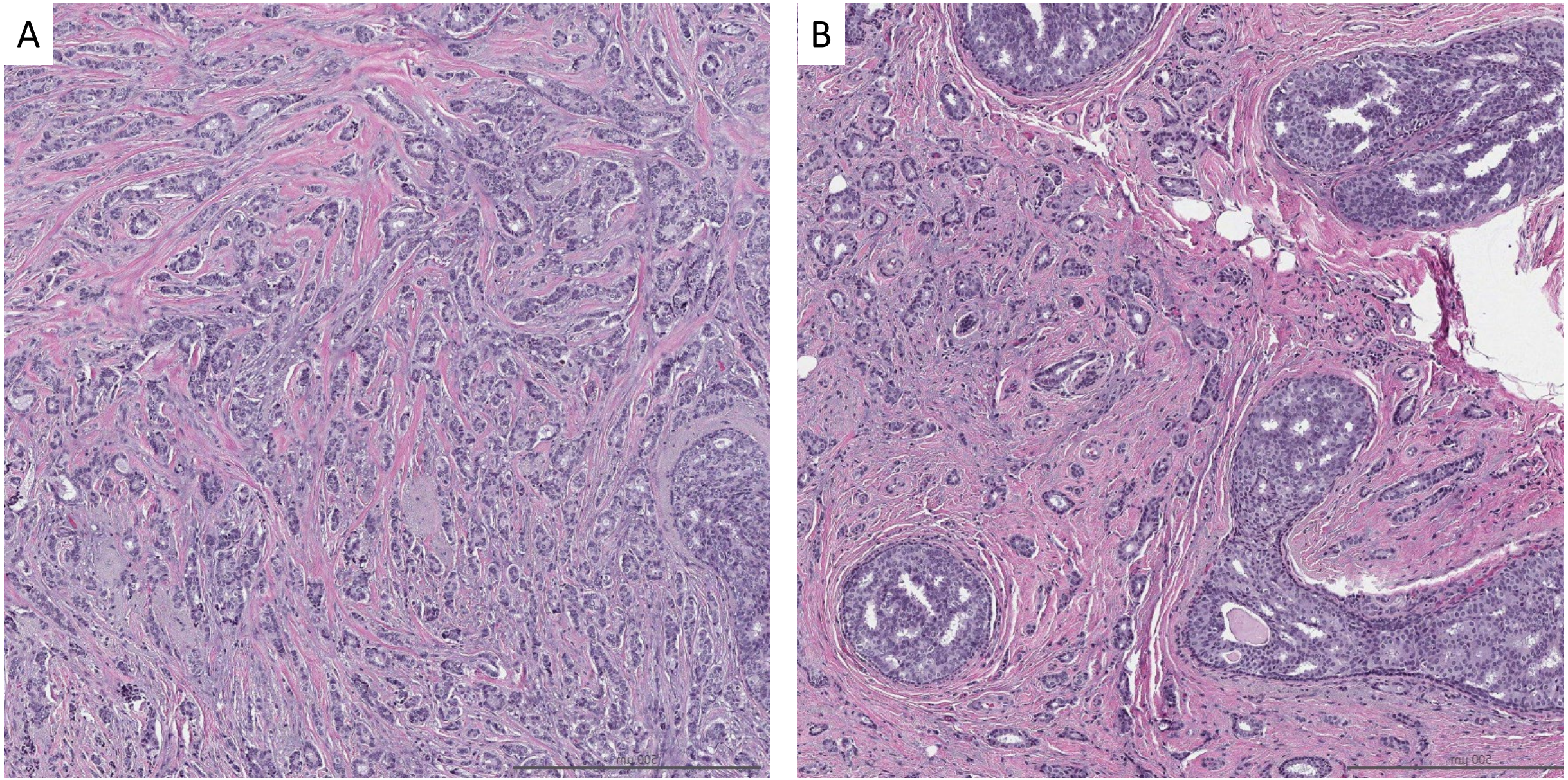

RSClin™ Educational Tool
User Input
18
Oncotype DX
Breast Recurrence Score® Result
Tumor Size (cm): 3.0
Tumor Grade (Differentiation): 2
Planned Hormonal Treatment: Tamoxifen
Patient Age At Surgery: 48
Calculation Estimates
When patient specific characteristics are added to the Oncotype DX Breast Recurrence Score result, the following risk estimates provide additional information on your patient:
Individualized distant recurrence risk at 10 years 12% (95% CI: 9% – 15%)
Individualized absolute chemotherapy benefit 1% (95% CI: -4% – 5%)


RSClin™ Educational Tool
User Input
18
Oncotype DX
Breast Recurrence Score® Result
Tumor Size (cm): 1.5
Tumor Grade (Differentiation): 3
Planned Hormonal Treatment: Tamoxifen
Patient Age At Surgery: 48
Calculation Estimates
When patient specific characteristics are added to the Oncotype DX Breast Recurrence Score result, the following risk estimates provide additional information on your patient:
Individualized distant recurrence risk at 10 years 13% (95% CI: 10% – 18%)
Individualized absolute chemotherapy benefit 5% (95% CI: 0% – 10%)


RSClin™ Educational Tool
User Input
18
Oncotype DX
Breast Recurrence Score® Result
Tumor Size (cm): 1.5
Tumor Grade (Differentiation): 2
Planned Hormonal Treatment: Tamoxifen
Patient Age At Surgery: 48
Calculation Estimates
When patient specific characteristics are added to the Oncotype DX Breast Recurrence Score result, the following risk estimates provide additional information on your patient:
Individualized distant recurrence risk at 10 years 7% (95% CI: 5% – 9%)
Individualized absolute chemotherapy benefit 1% (95% CI: -2% – 3%)

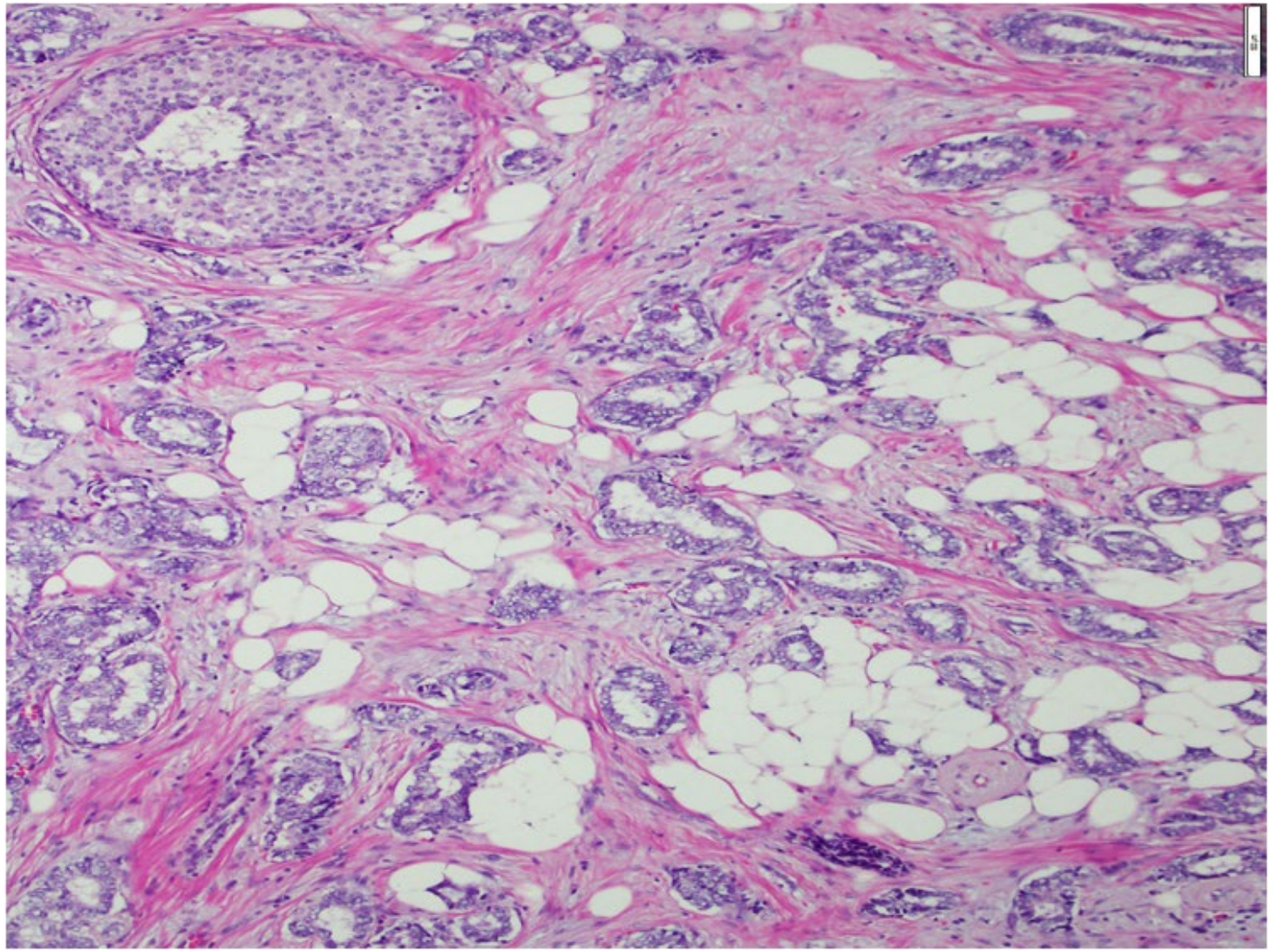
Guidelines for selecting sample for multigene assays

- Tumor content:
  - Choose the block with the greatest amount of invasive carcinoma and the least amount of non-invasive mammary epithelium (in situ carcinoma, hyperplastic epithelium, normal epithelium)
- If there is intratumoral heterogeneity, choose the block with the highest grade
- **Avoid biopsy cavity**


A
B

Case example

- 55-year-old woman
- Invasive ductal carcinoma, grade II, 6 mm
- ER 90%, PR 80%, HER2-negative (0)



RESULTS

Recurrence Score = 49

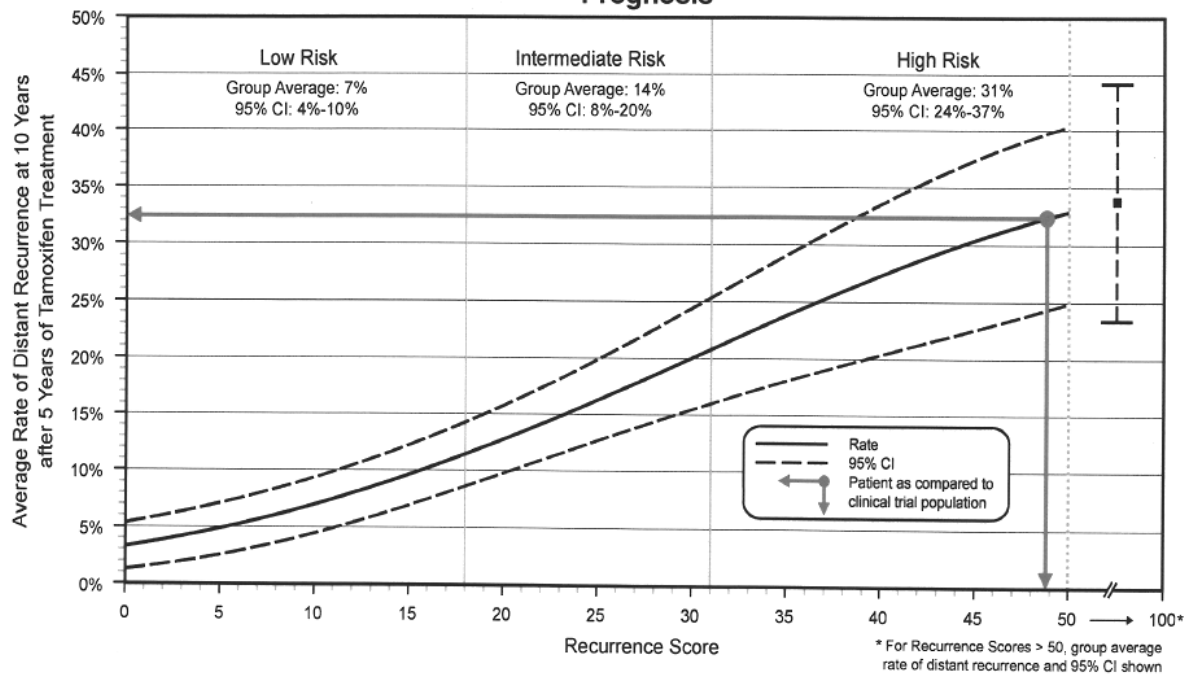
Test Results should be interpreted using the Clinical Experience information contained in this report which is derived from clinical studies involving patient populations with specific clinical features as noted in each section of the Clinical Experience. It is unknown whether the findings summarized in the Clinical Experience are applicable to patients with features different from those described.

CLINICAL EXPERIENCE: PROGNOSIS FOR NODE NEGATIVE, ER-POSITIVE PATIENTS

The Clinical Validation study included female patients with Stage I or II, Node Negative, ER-Positive breast cancer treated with 5 years of tamoxifen. Those patients who had a Recurrence Score of 49 had an Average Rate of Distant Recurrence of 32% (95% CI: 24%-40%)

The following results are from a clinical validation study of 668 patients from the NSABP B-14 study. *N Engl J Med* 2004; 351: 2817-26.

Recurrence Score vs Distant Recurrence in NODE NEGATIVE, ER-Positive Breast Cancer Prognosis



Node Negative

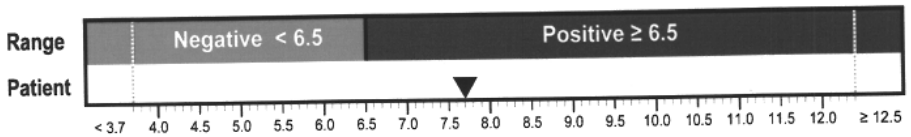
R33

QUANTITATIVE SINGLE GENE REPORT

The Onco*type* DX assay uses RT-PCR to determine the RNA expression of the genes below. These results may differ from ER, PR, or HER2 results reported using other methods or reported by other laboratories.[1]

The ER, PR, and HER2 Scores are also included in the calculation of the Recurrence Score.

ER Score = **7.7** **Positive**

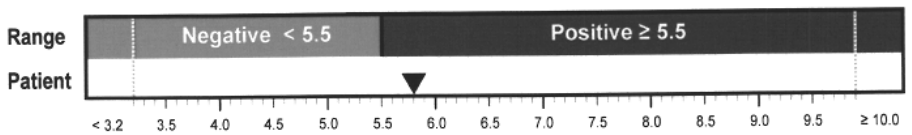


The ER Score positive/negative cut-off of 6.5 units was validated from a study of 761 samples using the 1D5 antibody (immunohistochemistry) and 607 samples using the SP1 antibody (immunohistochemistry). The standard deviation for the ER Score is less than 0.5 units.[2]

Clinical Experience:

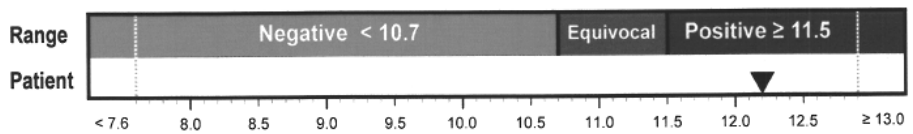
For ER positive breast cancer, the magnitude of tamoxifen benefit increases as the ER Score increases from 6.5 to ≥12.5.[3]
Please note: The Average Rate of Distant Recurrence reported on Page 1 based on the Recurrence Score was determined in patients who received 5 years of tamoxifen treatment and takes into account the magnitude of tamoxifen benefit indicated by the ER Score.

PR Score = **5.8** **Positive**

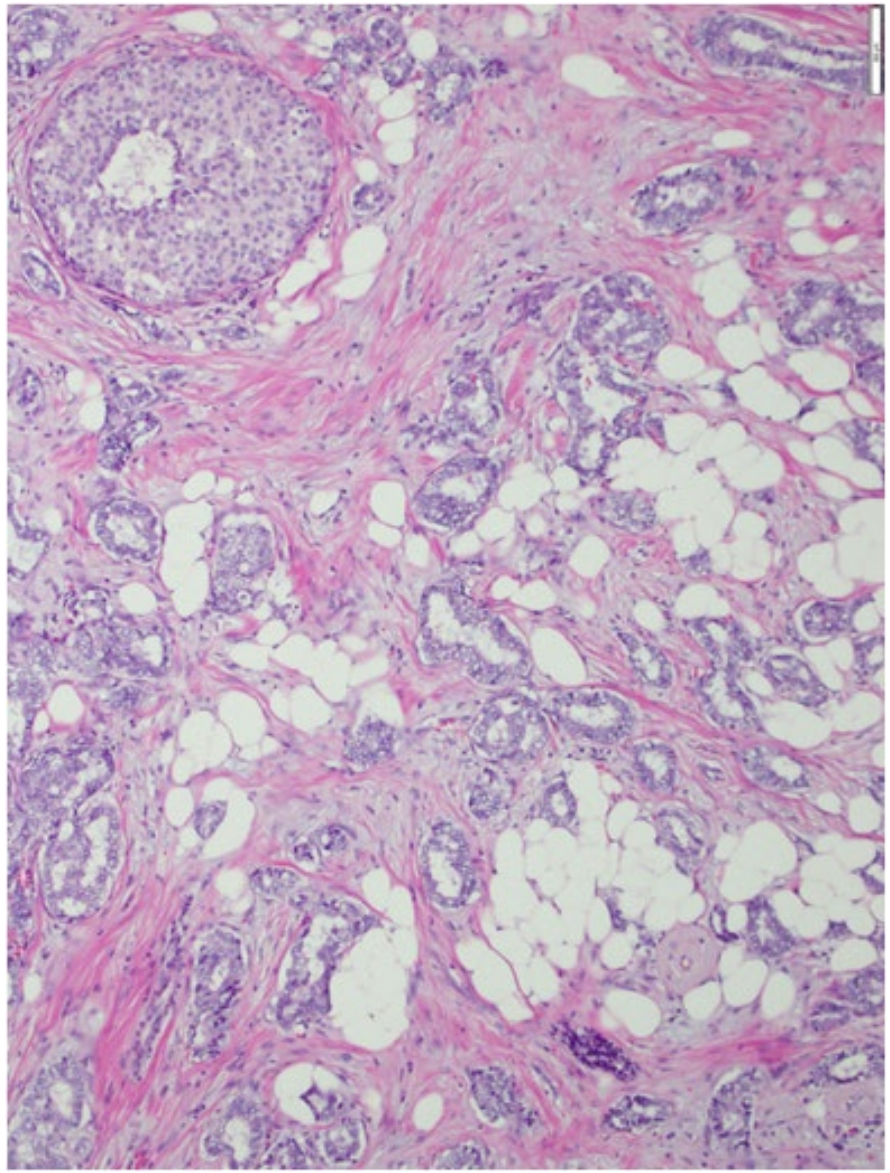


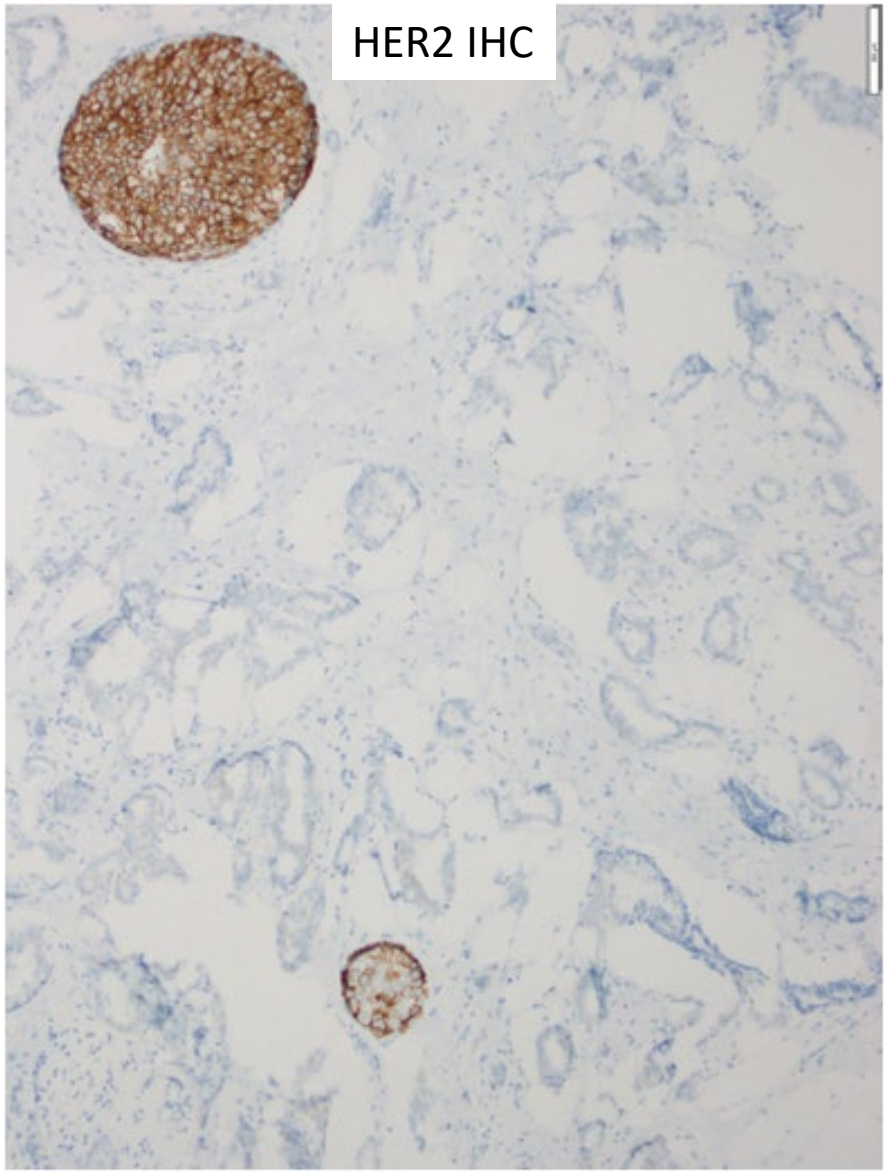
The PR Score positive/negative cut-off of 5.5 units was validated from a study of 761 samples using the PR636 antibody (immunohistochemistry) and another study of 607 samples using the PR636 antibody (immunohistochemistry). The standard deviation for the PR Score is less than 0.5 units.[2]

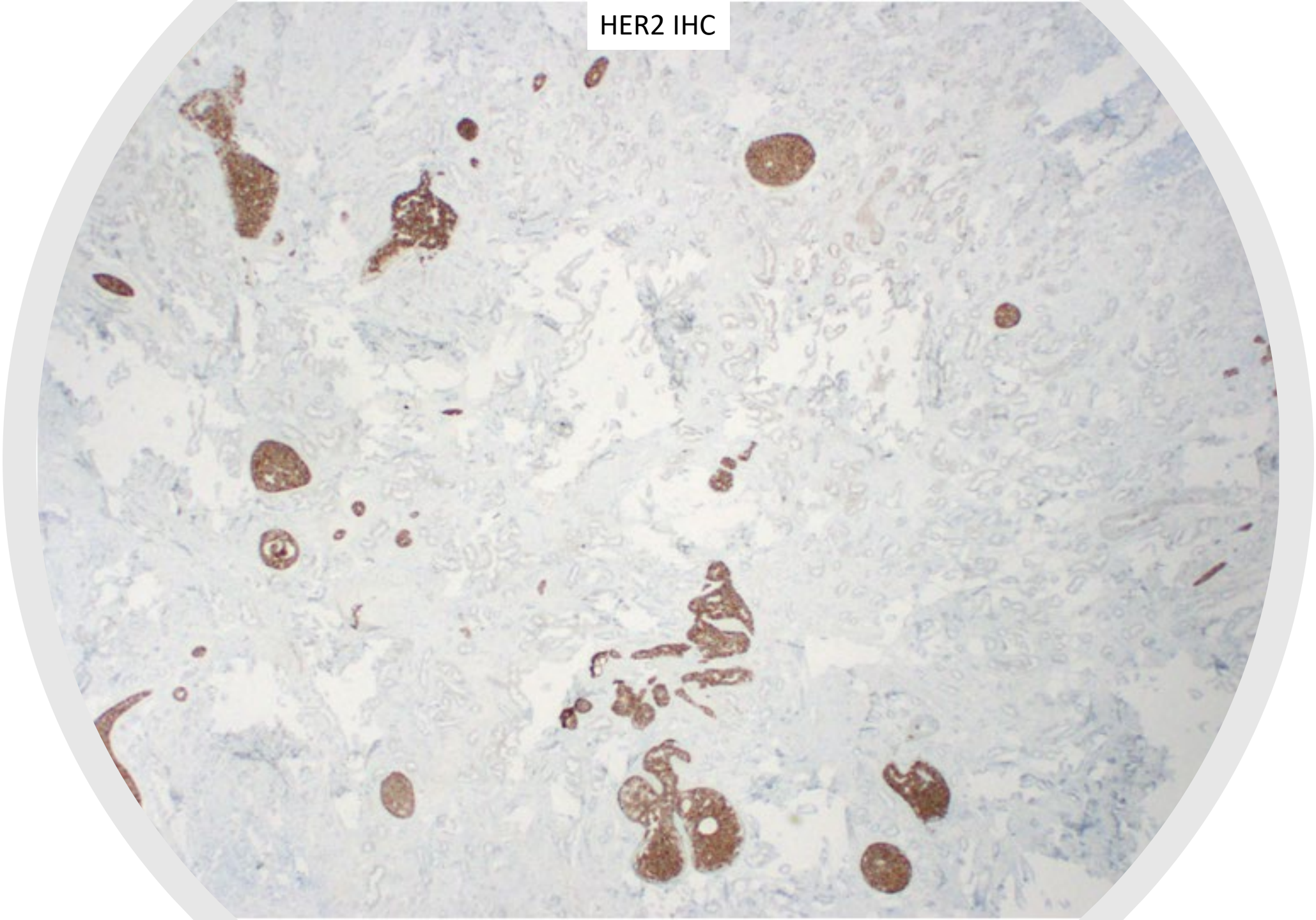
HER2 Score = **12.2** **Positive**

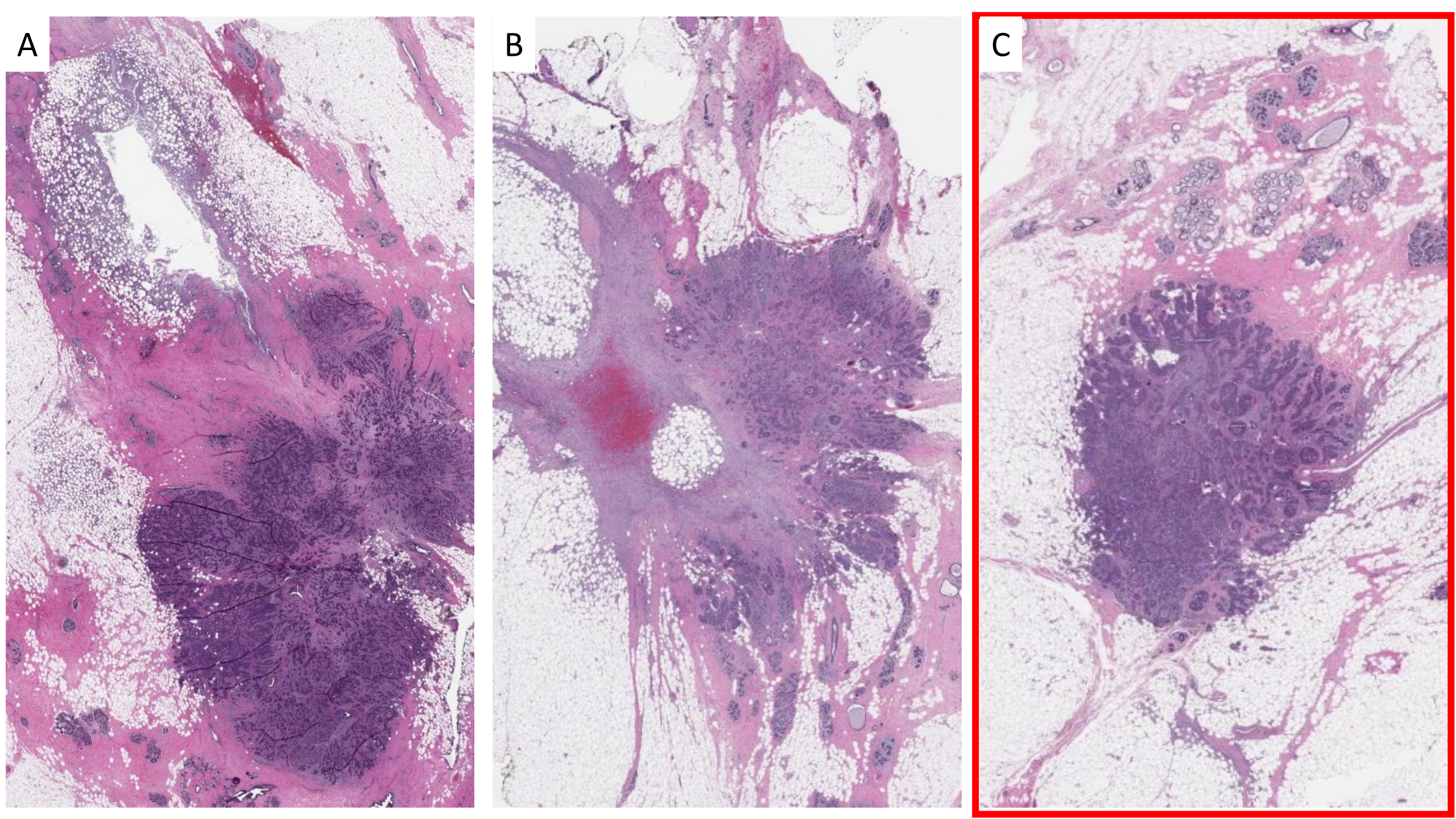


The HER2 positive cut-off of ≥ 11.5 units, equivocal range from 10.7 to 11.4 units, and negative cut-off of < 10.7 units were validated from concordance studies of 755 samples using the HercepTest™ assay (immunohistochemistry) and another study of 568 samples using the PathVysion® assay (FISH). The standard deviation for the HER2 score is less than 0.5 units.[4]




HER2 IHC


HER2 IHC


A
B
C

Biopsy Cavities in Breast Cancer Specimens: Their Impact on Quantitative RT-PCR Gene Expression Profiles and Recurrence Risk Assessment

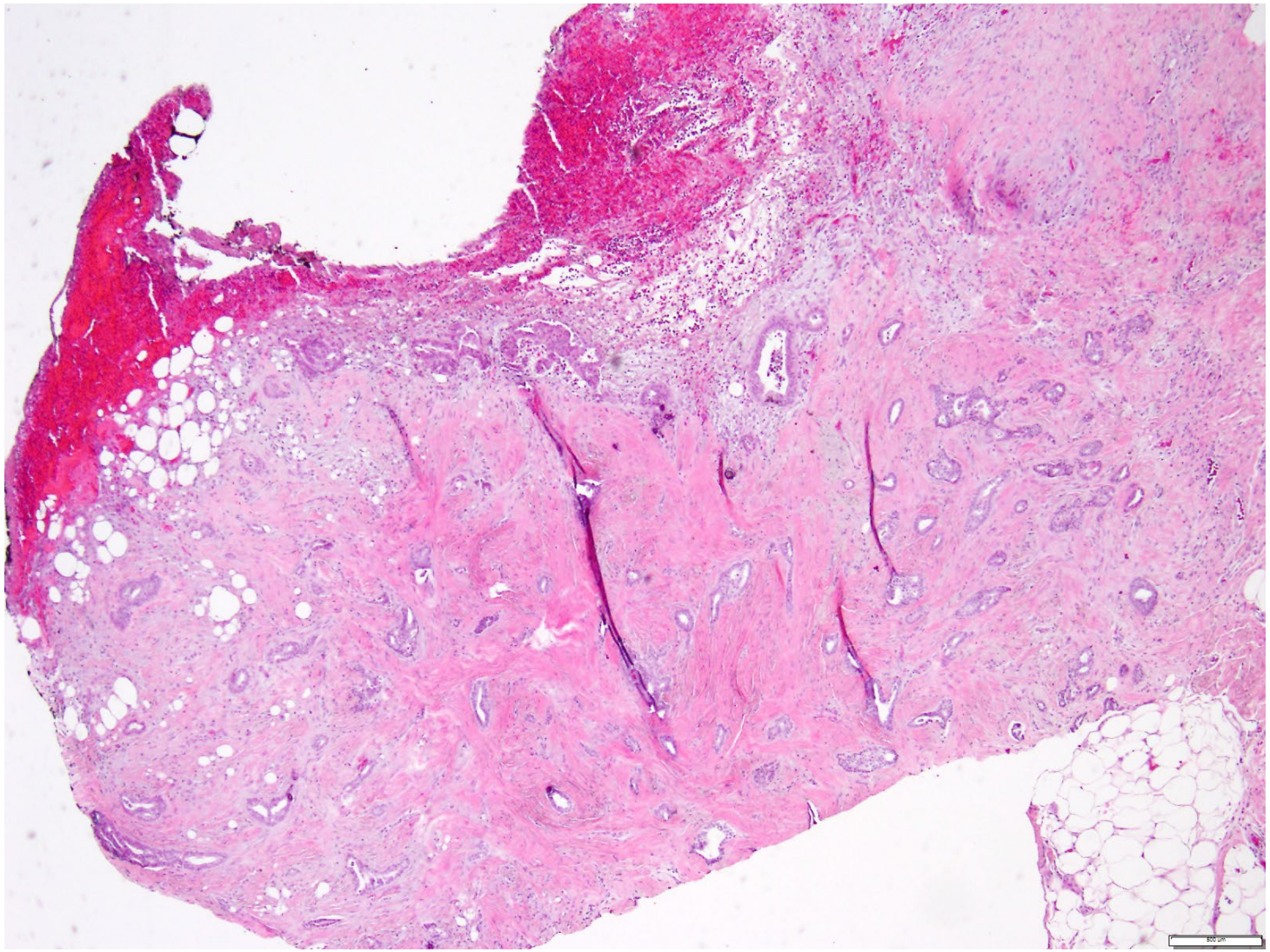
Author(s): F.L. Baehner, C. Quale, D. Cherbavaz, C. Sangli, C. Pomeroy, A. Chen, F. Lane, L. Intagliatta, A. Goddard, S. Shak; University of California, San Francisco, Redwood City, CA; Genomic Health, Redwood City, CA

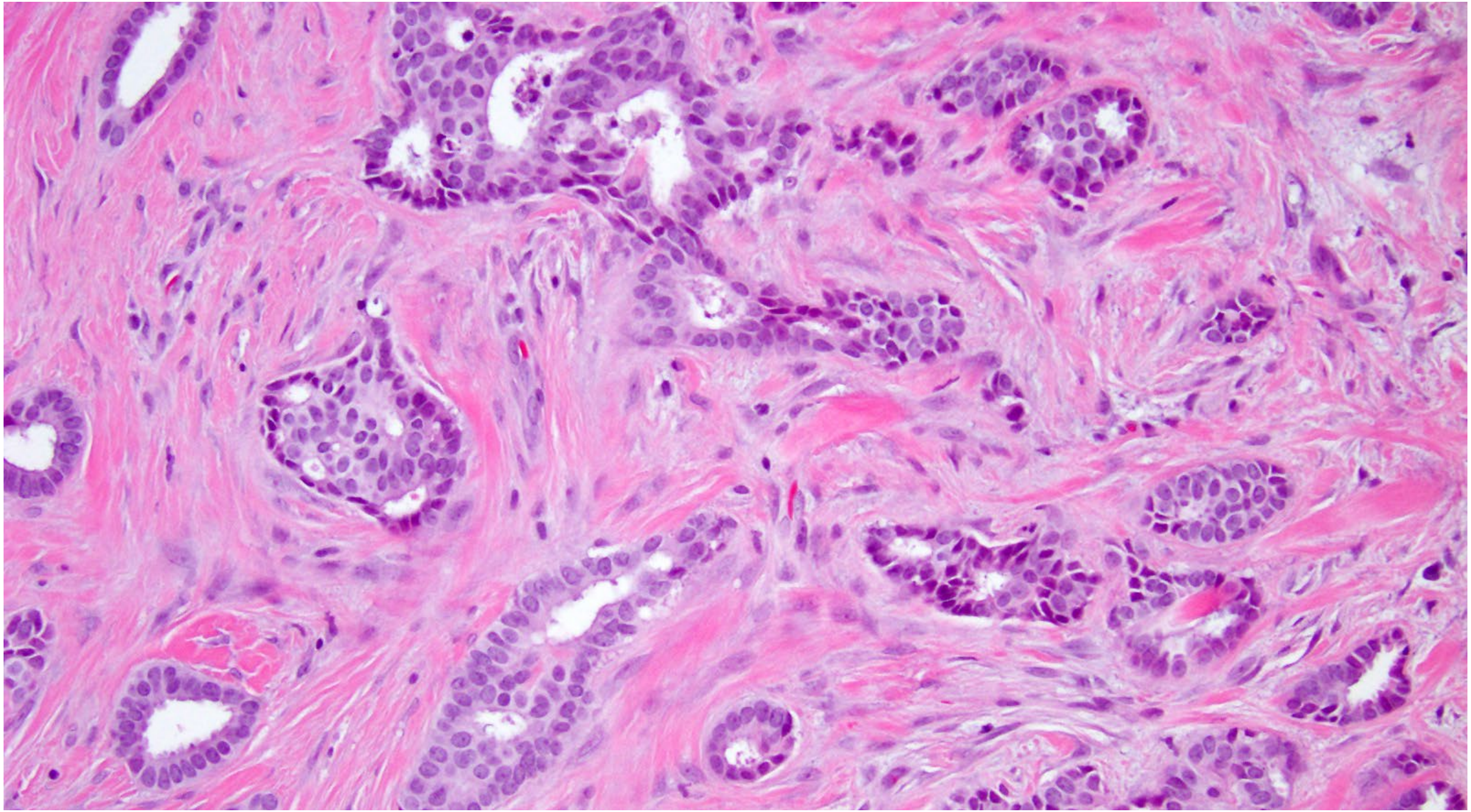
Baehner FL et al. USCAP 2009

- 48 invasive breast carcinomas (15 well, 18 moderate, and 15 poorly diff)
- 21-gene RT-PCR assay
  - Whole sections (WS, contains BxC)
  - Enriched tumor (ET, BxC excluded by manual microdissection)
- Statistically significant differences 6 of the 16 cancer-related genes:
  - BAG1 , CD68, ER, GSTM1, STK15, and STMY3
- Expression of CD68 was higher and ER was lower in WS containing BxC
- The inclusion of BxC in breast cancer specimens can impact RS

Case example

- 69- year-old woman, invasive ductal carcinoma, grade I, 3.5 mm, ER 95%, PR 50%, HER2-negative (0)





BREAST CANCER ASSAY DESCRIPTION

Oncotype DX Breast Cancer Assay uses RT-PCR to determine the expression of a panel of 21 genes in tumor tissue. The Recurrence Score® is calculated from the gene expression results. The Recurrence Score range is from 0-100.

RESULTS

Breast Cancer Recurrence Score = 34

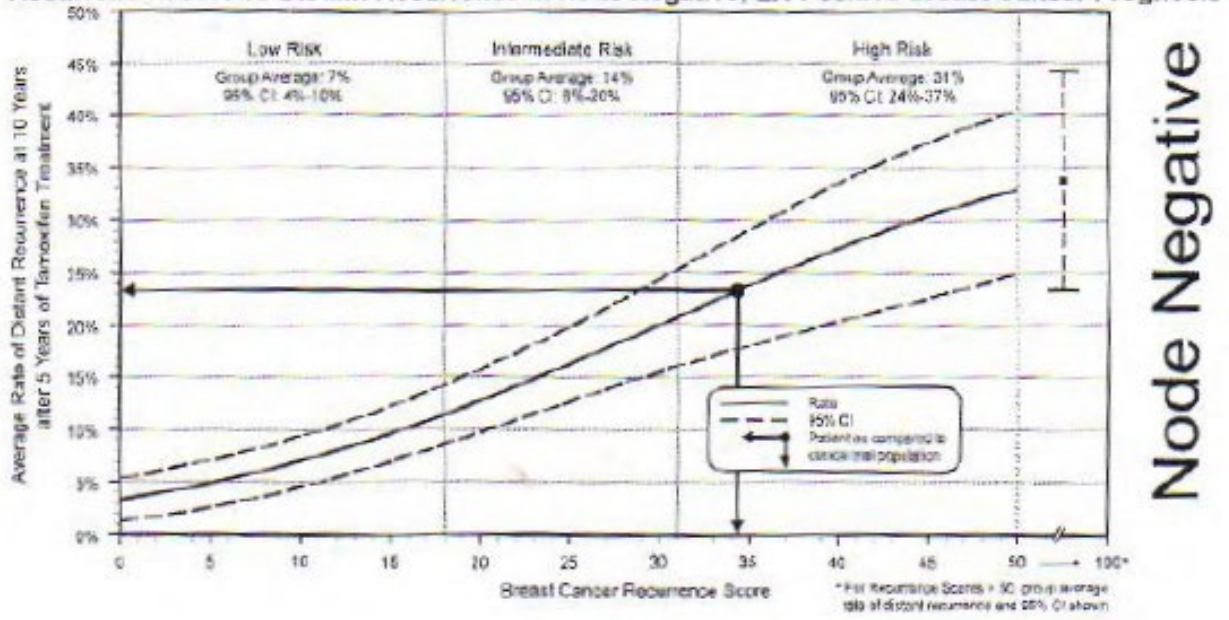
The findings summarized in the Clinical Experience sections of this report are applicable to the patient populations defined in each section. It is unknown whether the findings apply to patients outside these criteria.

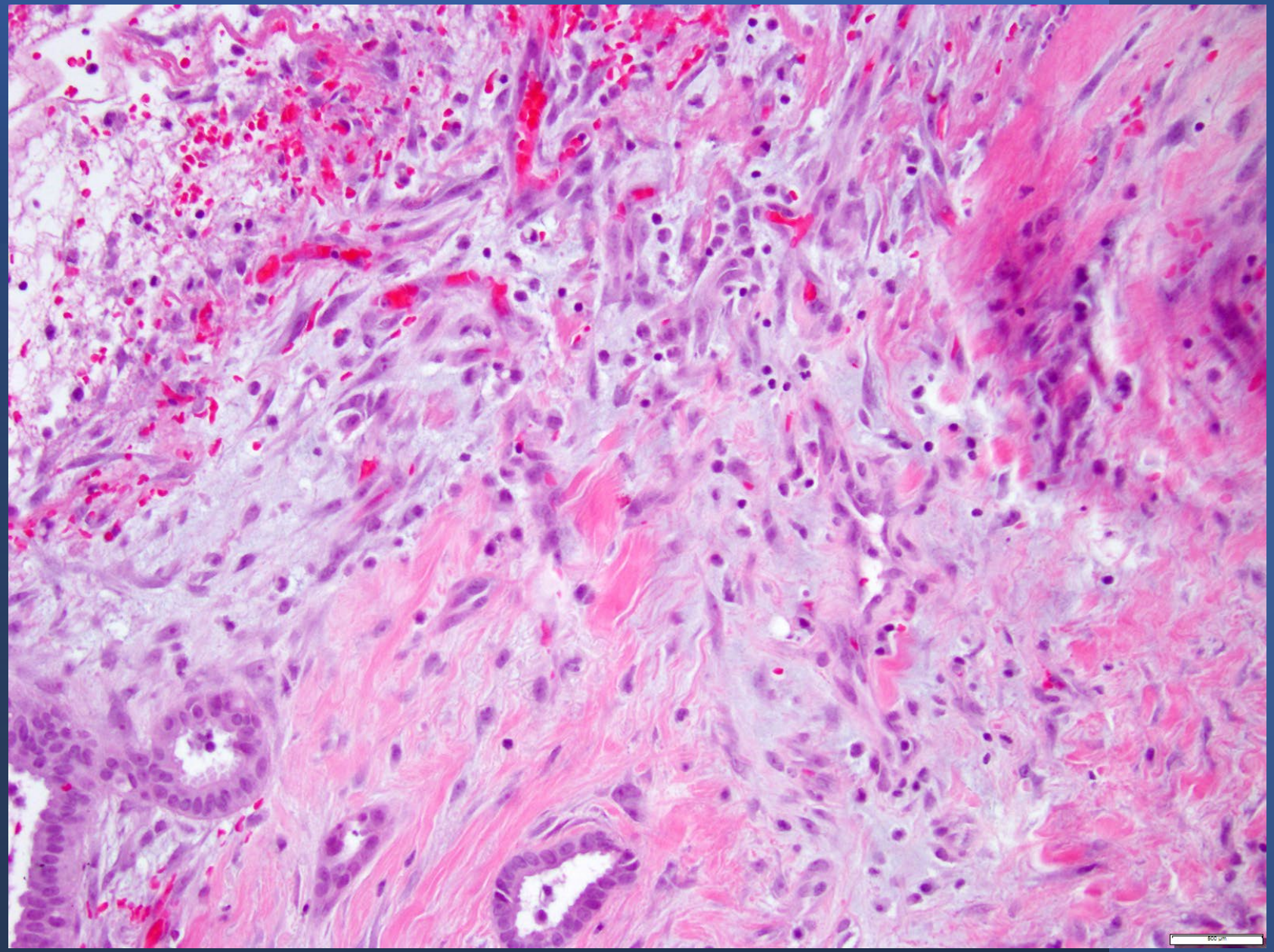
CLINICAL EXPERIENCE: PROGNOSIS FOR NODE NEGATIVE, ER-POSITIVE PATIENTS

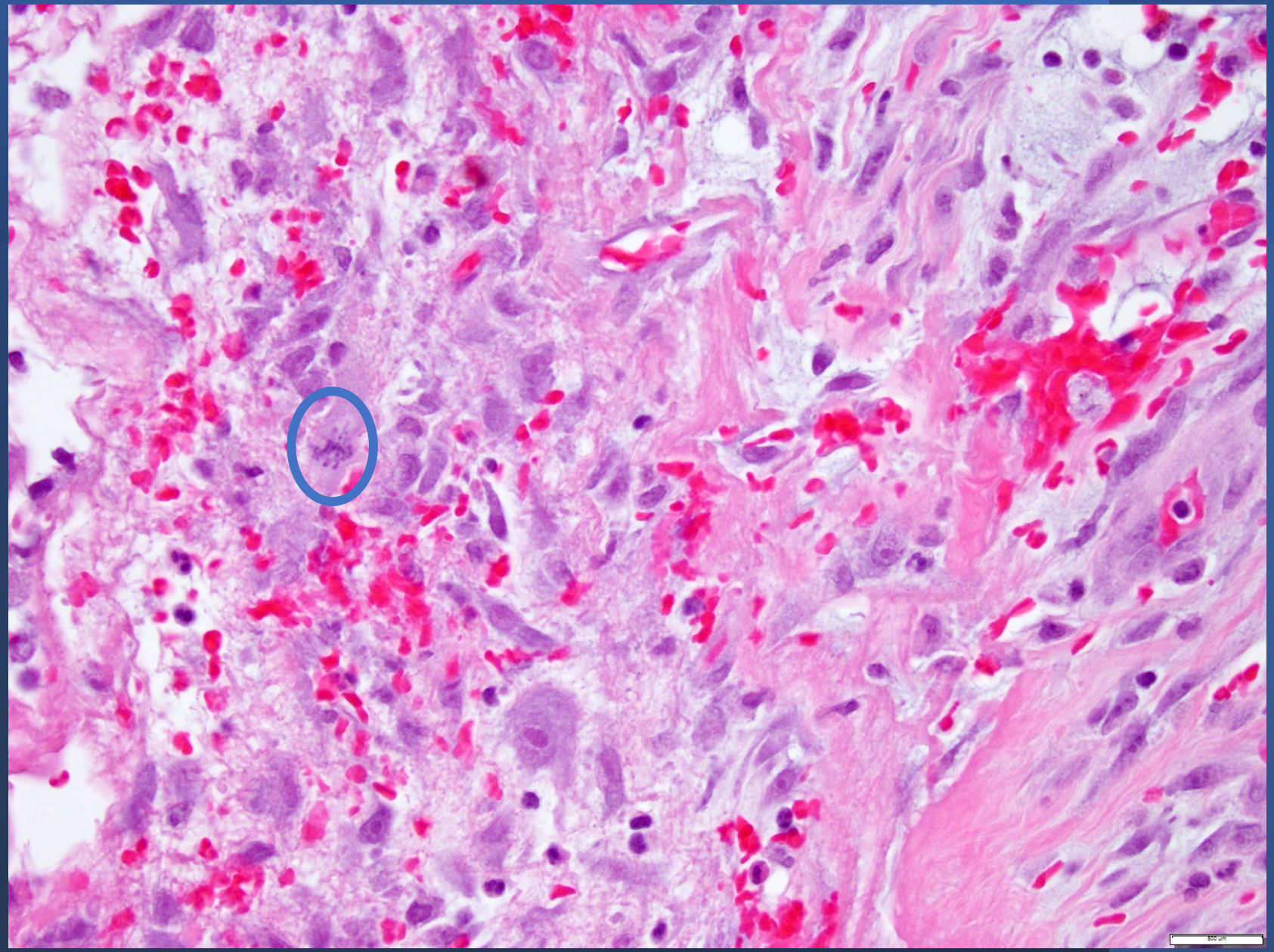
The Clinical Validation study included female patients with Stage I or II, Node Negative, ER-Positive breast cancer treated with 5 years of tamoxifen. Those patients who had a Recurrence Score of 34 had an Average Rate of Distant Recurrence of **23% (95% CI: 18%-28%)**

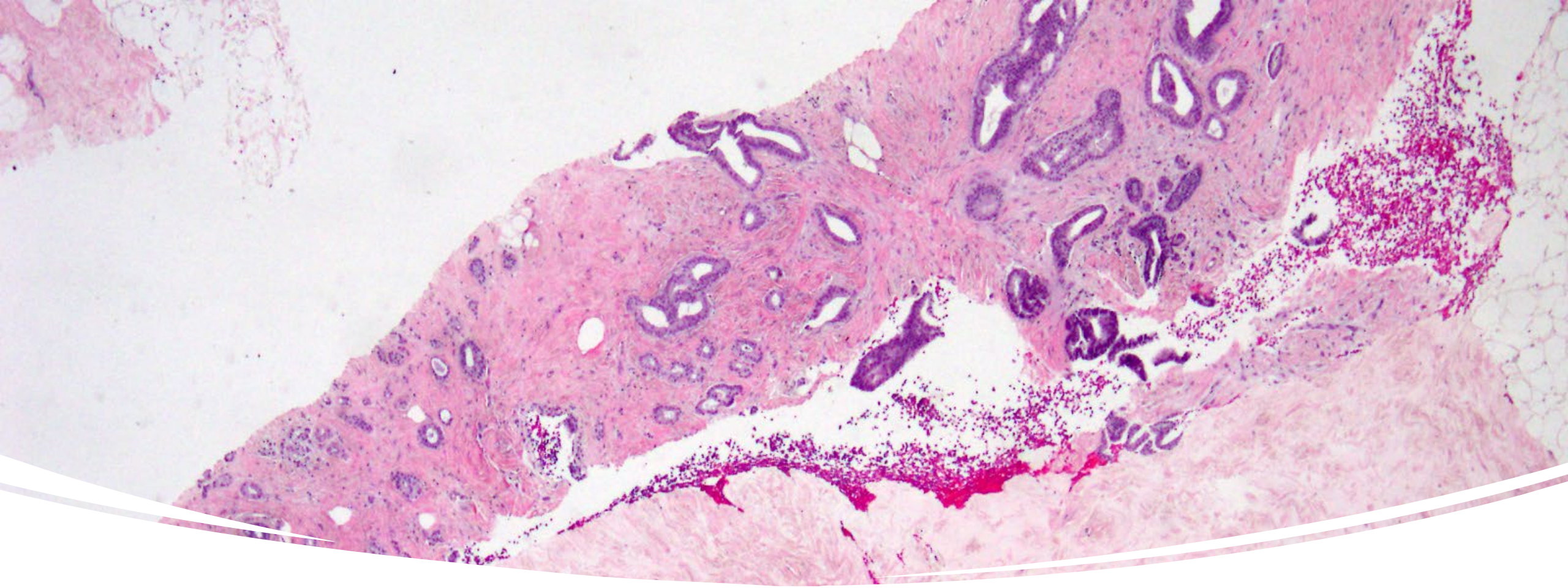
The following results are from a clinical validation study of 668 patients from the NSABP B-14 study. *N Engl J Med* 2004; 351: 2817-26.

Recurrence Score vs Distant Recurrence in Node Negative, ER-Positive Breast Cancer Prognosis









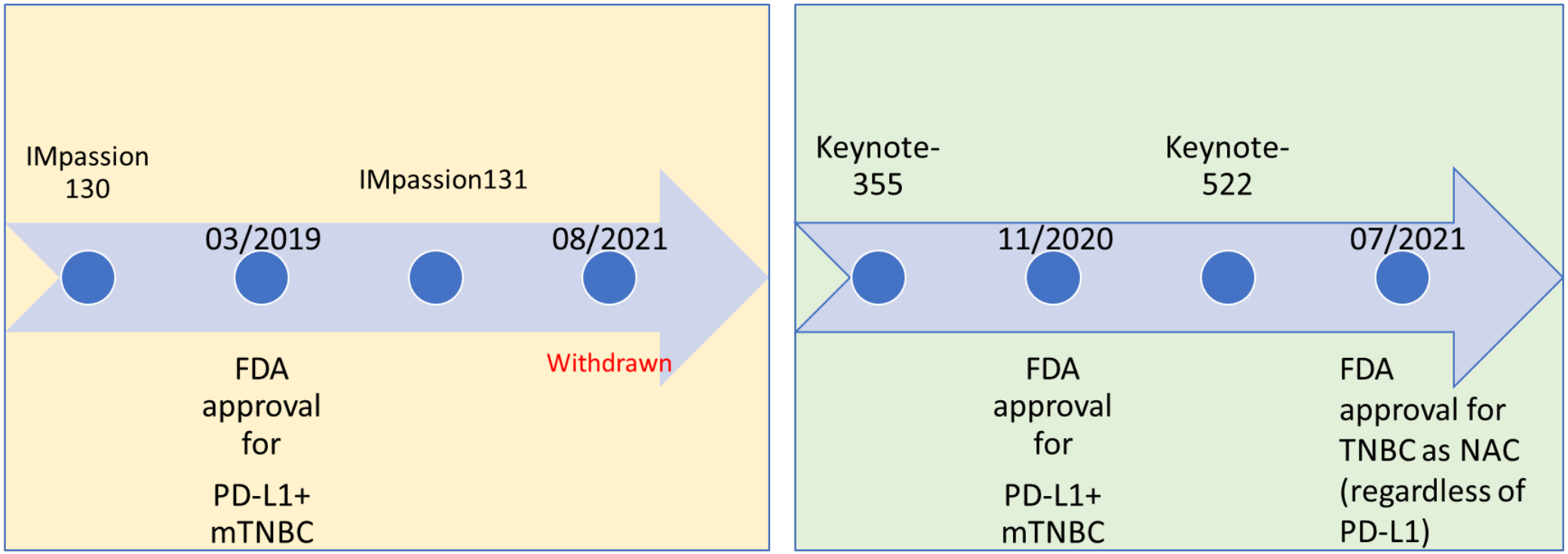
Re-tested on core biopsy

- **Recurrence score = 8**
- **RT and Arimidex. No chemotherapy**
- **NED (Follow-up 140 months)**

PD-L1

FDA approved immune checkpoint inhibitors for breast cancer (TNBC)

- Atezolizumab (anti-PD-L1)
- Pembrolizumab (anti-PD1)

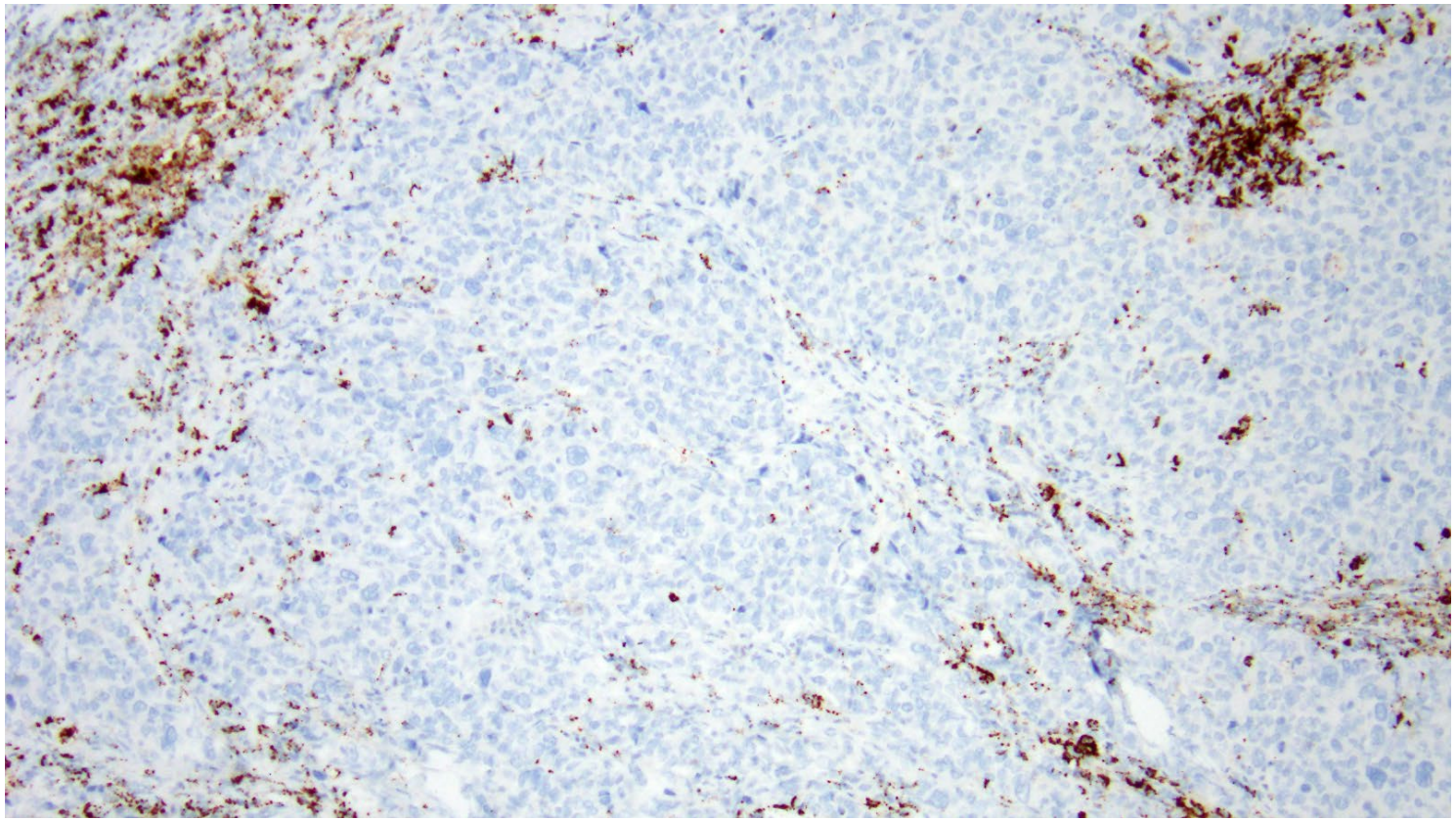


FDA approved companion diagnostic assays for PD-L1 in breast cancer

	SP142	22C3 pharmDx
Immunotherapy	Atezolizumab	Pembrolizumab
Platform	Ventana BenchMark	DAKO
Scoring methods	Immune cells (IC)	Combined positive score (CPS)
Positivity definition	IC≥1%	CPS≥10
Clinical Trial	IMpassion 130	KEYNOTE-355
Breast cancer subtype	Locally advanced or metastatic TNBC*	Locally advanced or metastatic TNBC
Chemotherapy	Nab-paclitaxel	Taxane or gemcitabine-carboplatin

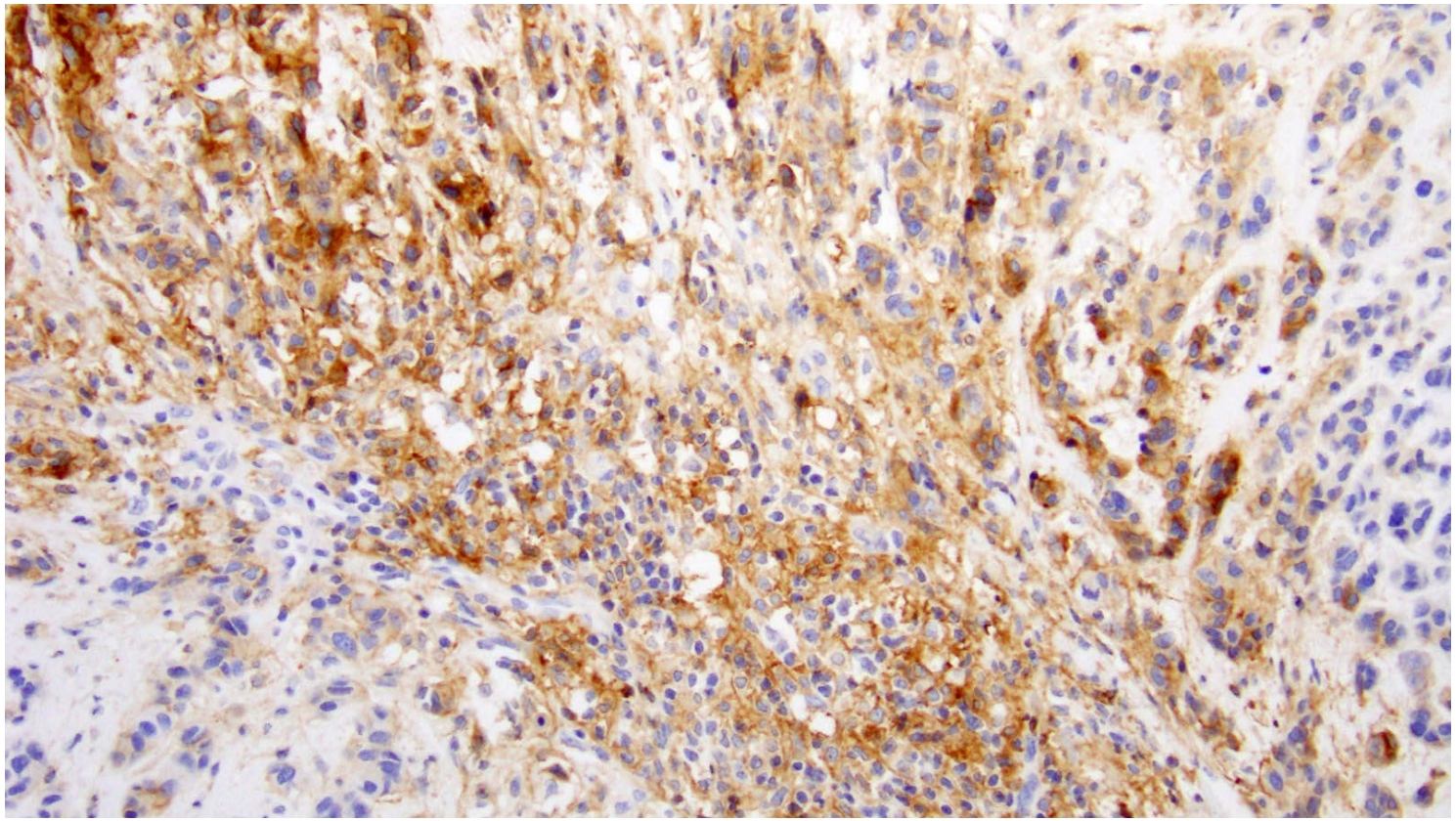
PD-L1 SP142

- PD-L1 expression in tumor infiltrating immune cells (IC) covering ≥1% of tumor area (IC ≥1%)



PD-L1 22C3

- Combined positive score (CPS)≥10

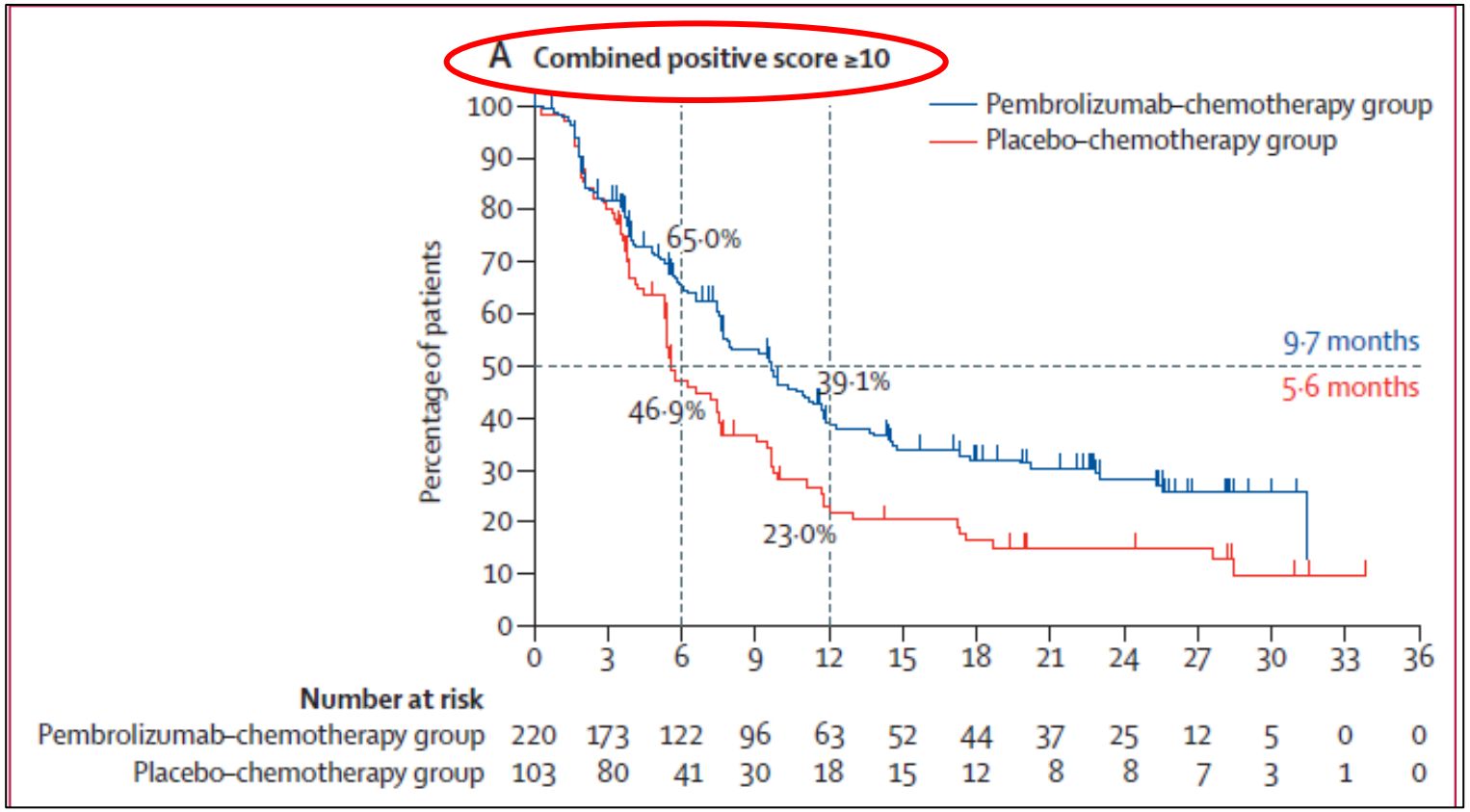


PD-L1 22C3: combined positive score (CPS)

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# of viable tumor cells}} \times 100$$

Keynote-355

	n/N	Events	mPFS, mos	HR (95% CI)	*P* value (one-sided)
Pembro + CT	136/220	61.8%	9.7	0.65 (0.49-0.86)	0.0012
Placebo + CT	79/103	76.7%	5.6		

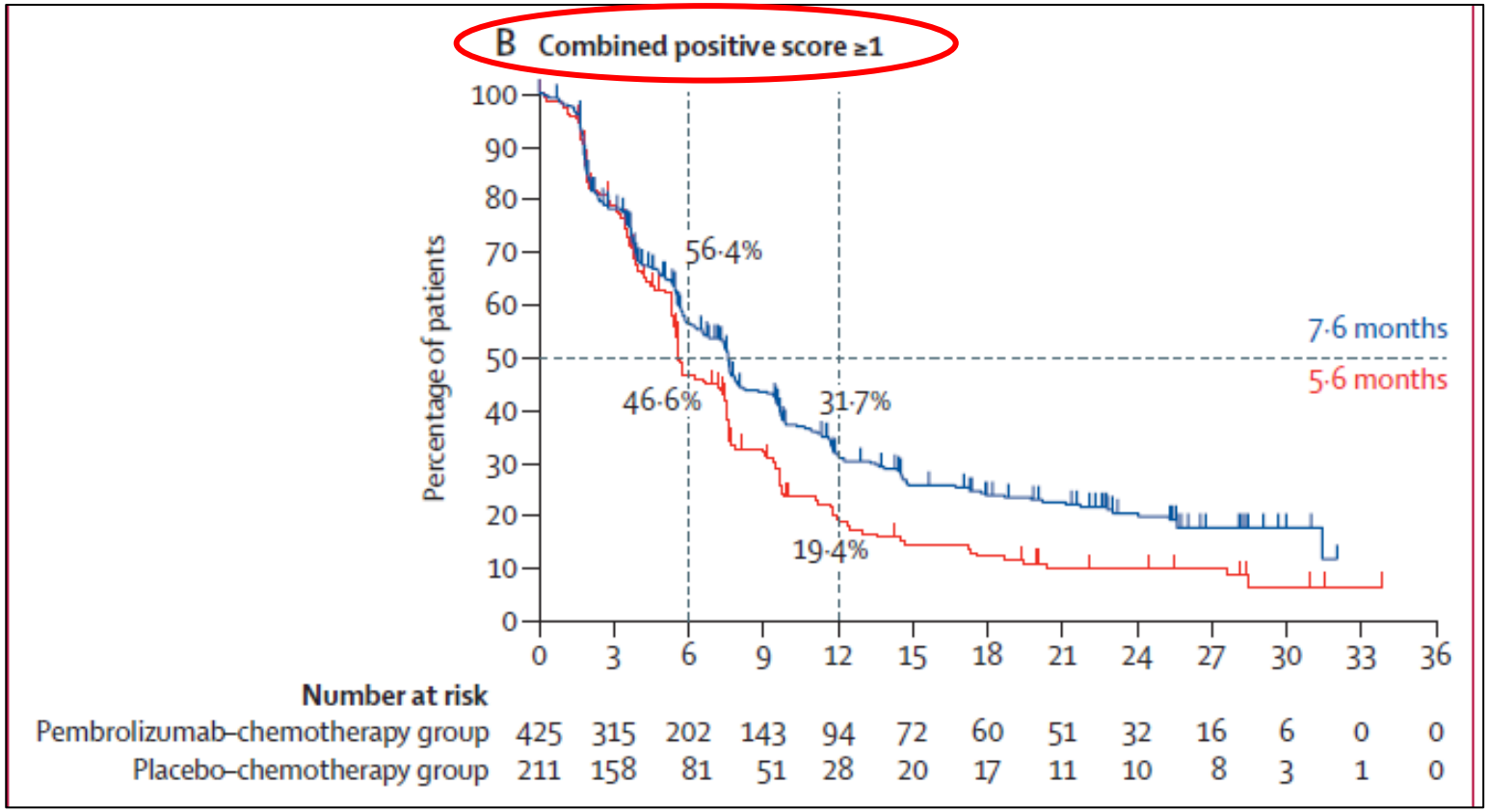


Cortes J et al. *Lancet*. 2020; 396:1817-1828

Keynote-355

	n/N	Events	mPFS, mos	HR (95% CI)	*P* value (one-sided)
Pembro + CT	288/425	67.8%	7.6	0.74 (0.61-0.90)	0.0014*
Placebo + CT	162/211	76.8%	5.6		

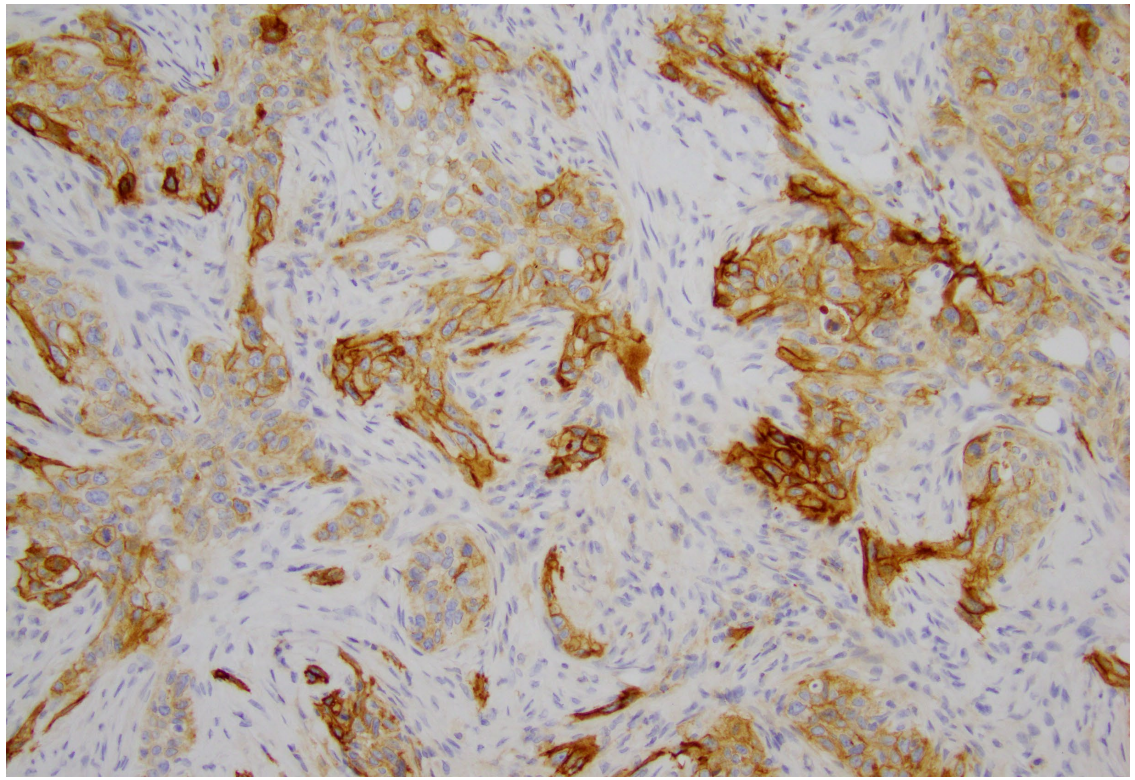
*No significant difference in PFS in CPS ≥1 according to the prespecified statistical criterion of alpha=0·00111

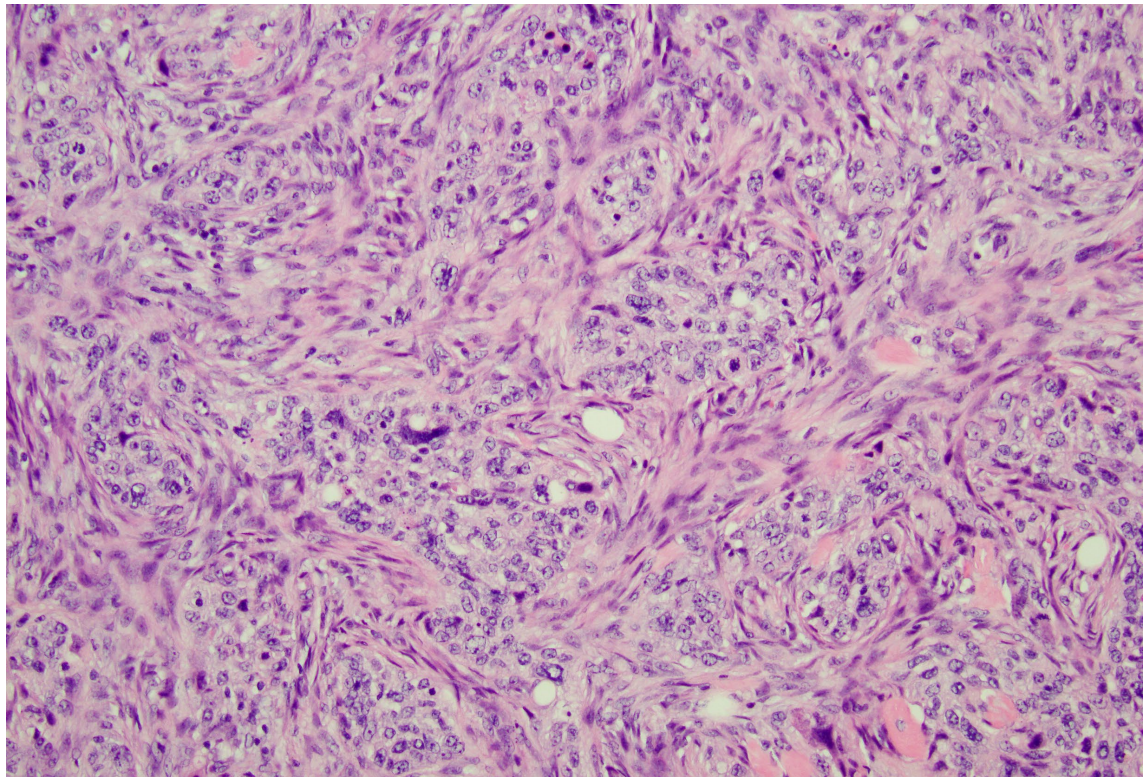


Cortes J et al. *Lancet*. 2020; 396:1817-1828

PD-L1 22C3 scoring criteria

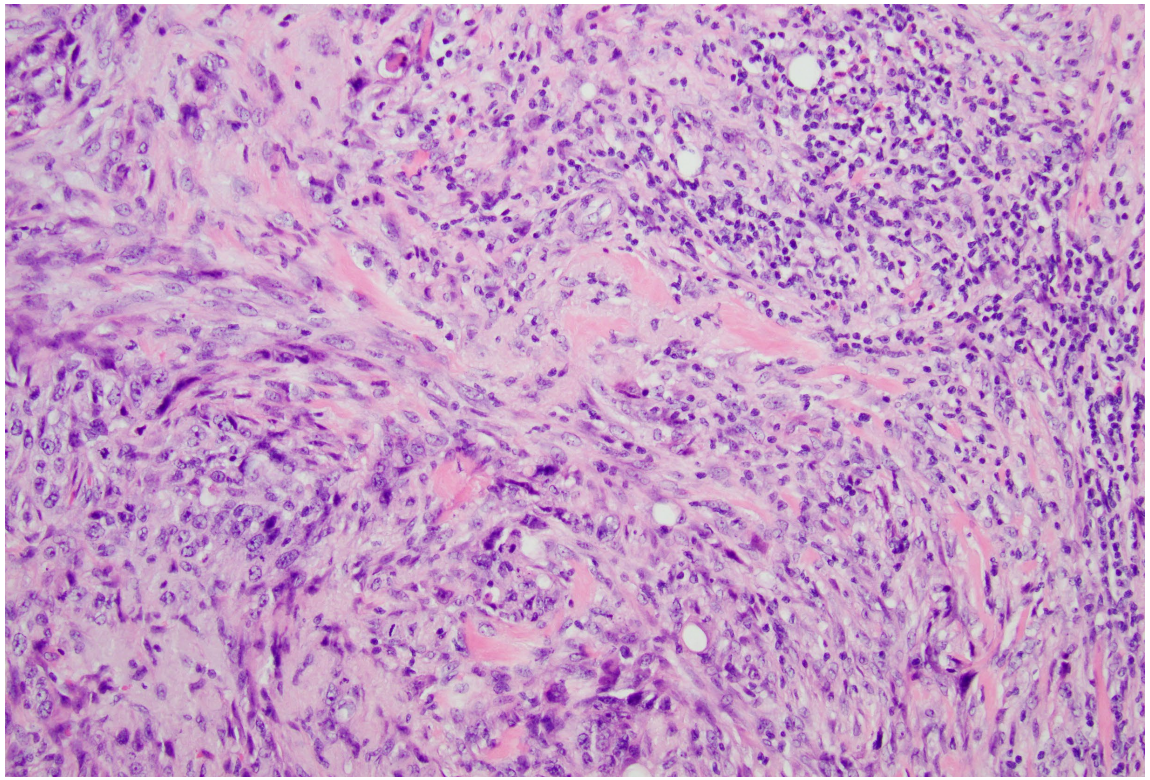
- Tumor cells: partial or complete *membrane staining* at any intensity

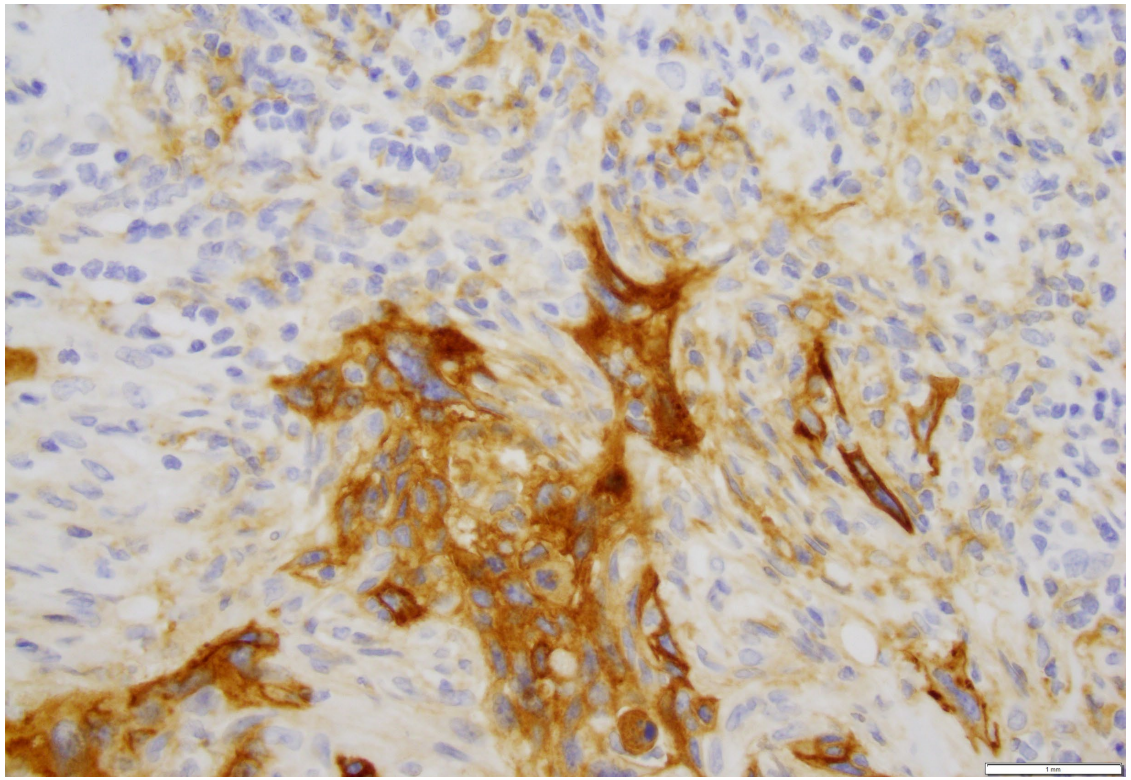




PD-L1 22C3 scoring criteria

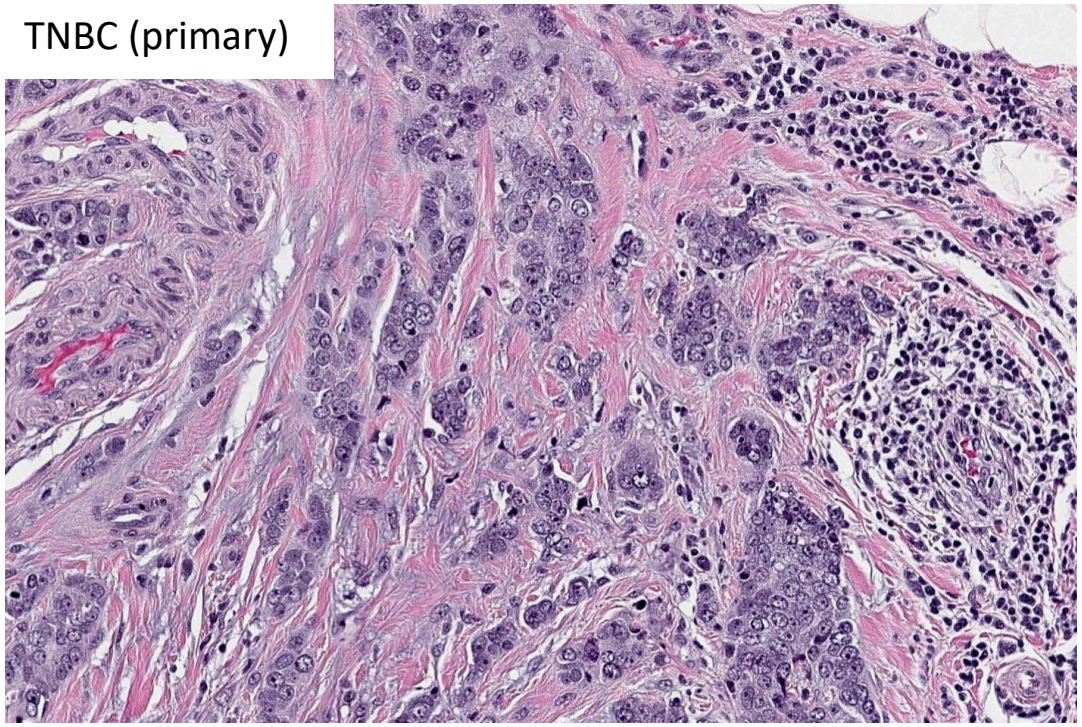
- Immune cells (*lymphocytes and macrophages)*:
  - *membrane and/or cytoplasmic staining*



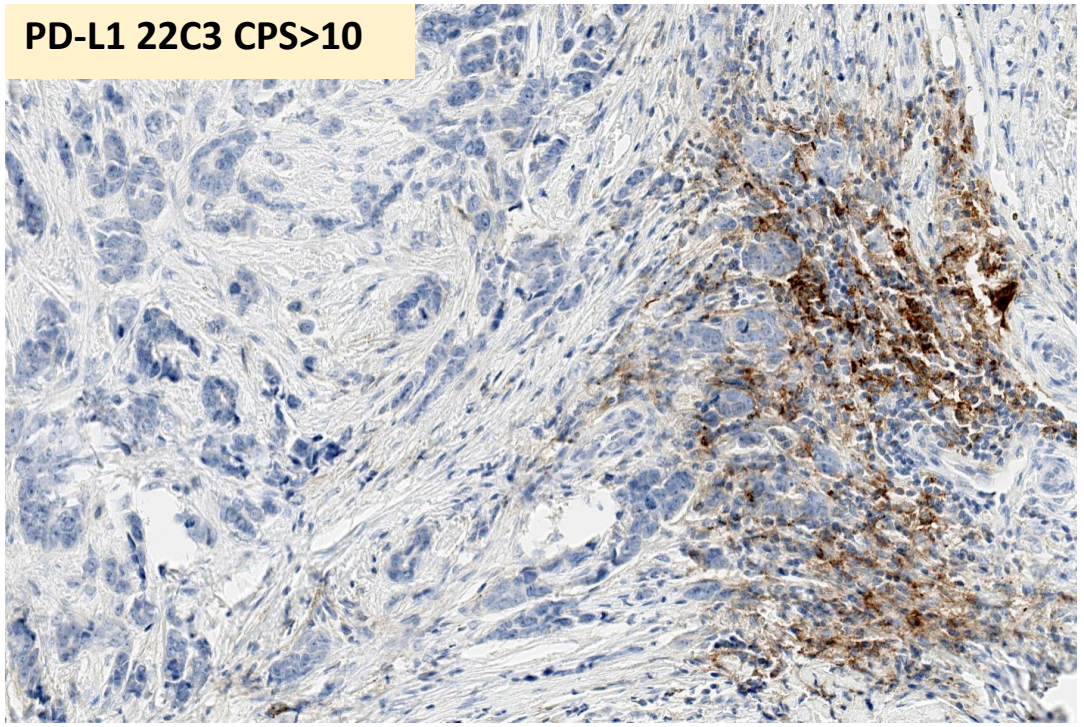


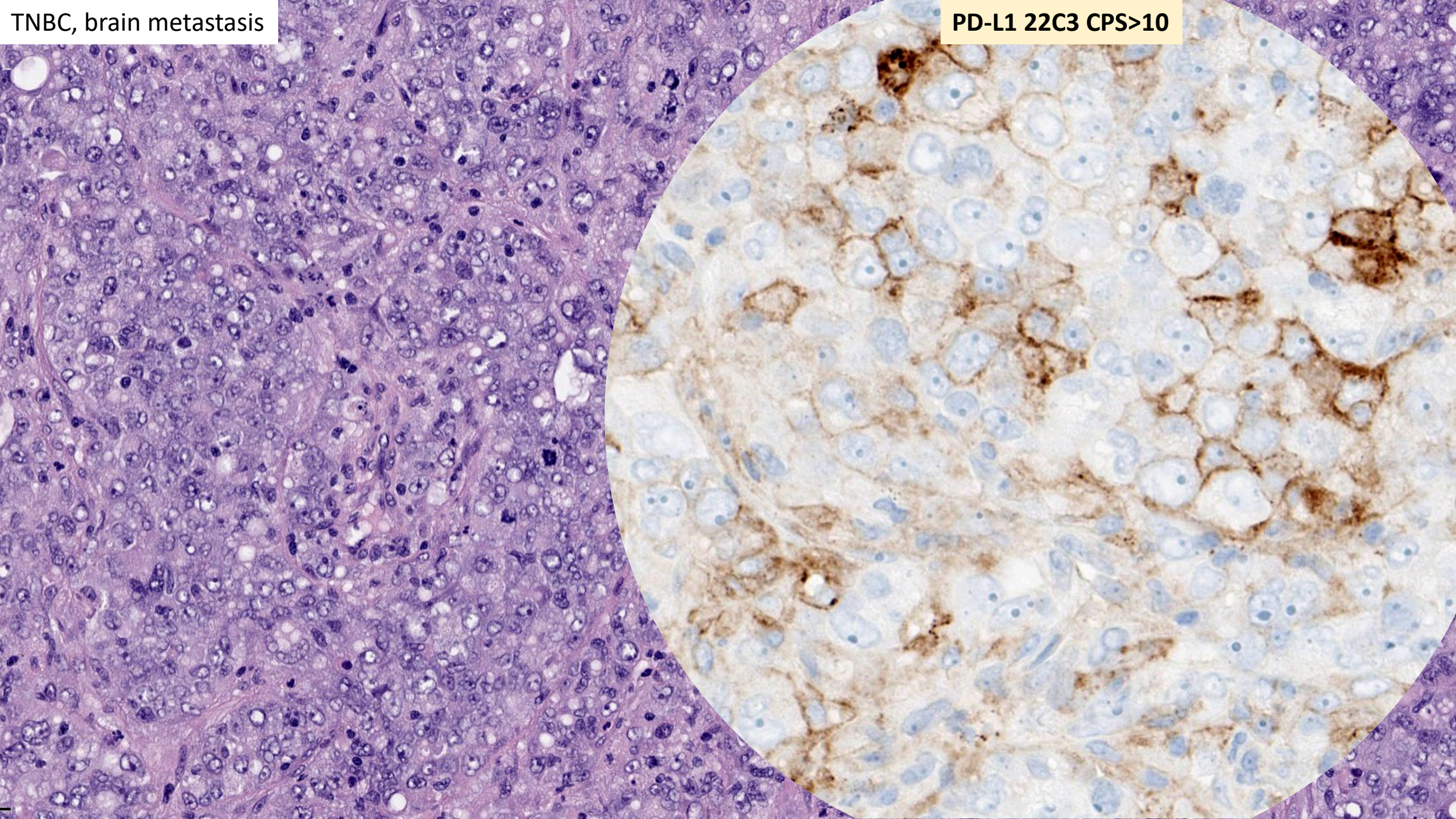
Examples

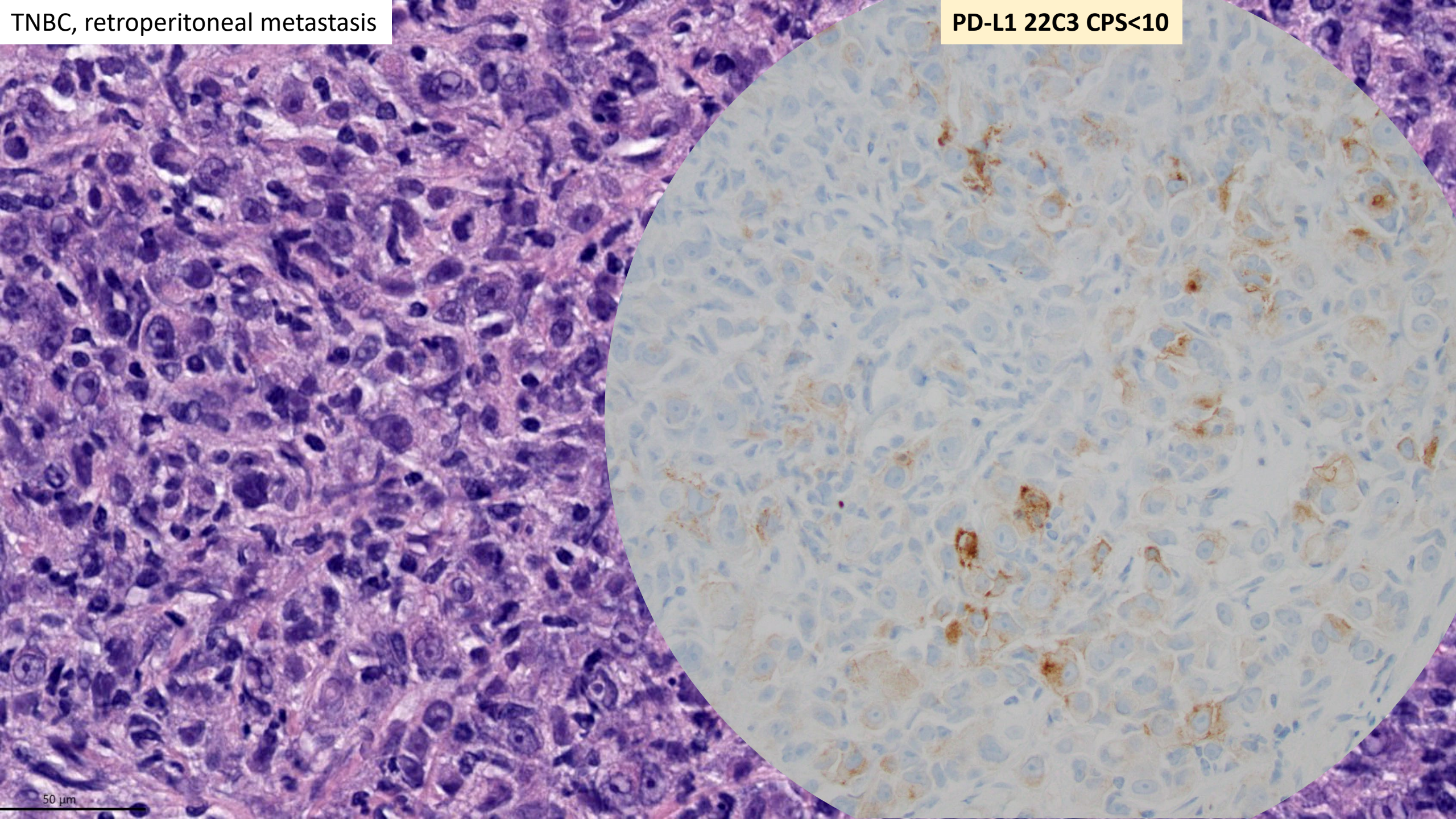
TNBC (primary)



PD-L1 22C3 CPS>10




TNBC, brain metastasis
PD-L1 22C3 CPS>10


TNBC, retroperitoneal metastasis
PD-L1 22C3 CPS<10
50 µm

Technical considerations

	SP142	22C3 pharmDx
Pre-analytic conditions	Fixation in 10% neutral buffered formalin for 6-72 hours	Fixation in 10% neutral buffered formalin for 12-72 hours
Cut slide stability	- Within 2 months of sectioning stored at room temperature - Within 4 months (5±3°C)	- Within 4 months of sectioning stored at room temperature - Within 7.5 months at 2–8 °C
Specimen adequacy	At least 50 viable tumor cells with associated stroma	A minimum of 100 viable tumor cells
Tissue samples	- Core biopsy or resection - Primary site or metastasis	- Core biopsy or resection - Primary site or metastasis

Specimens ***unacceptable*** for PD-L1 testing due to lack of validation studies

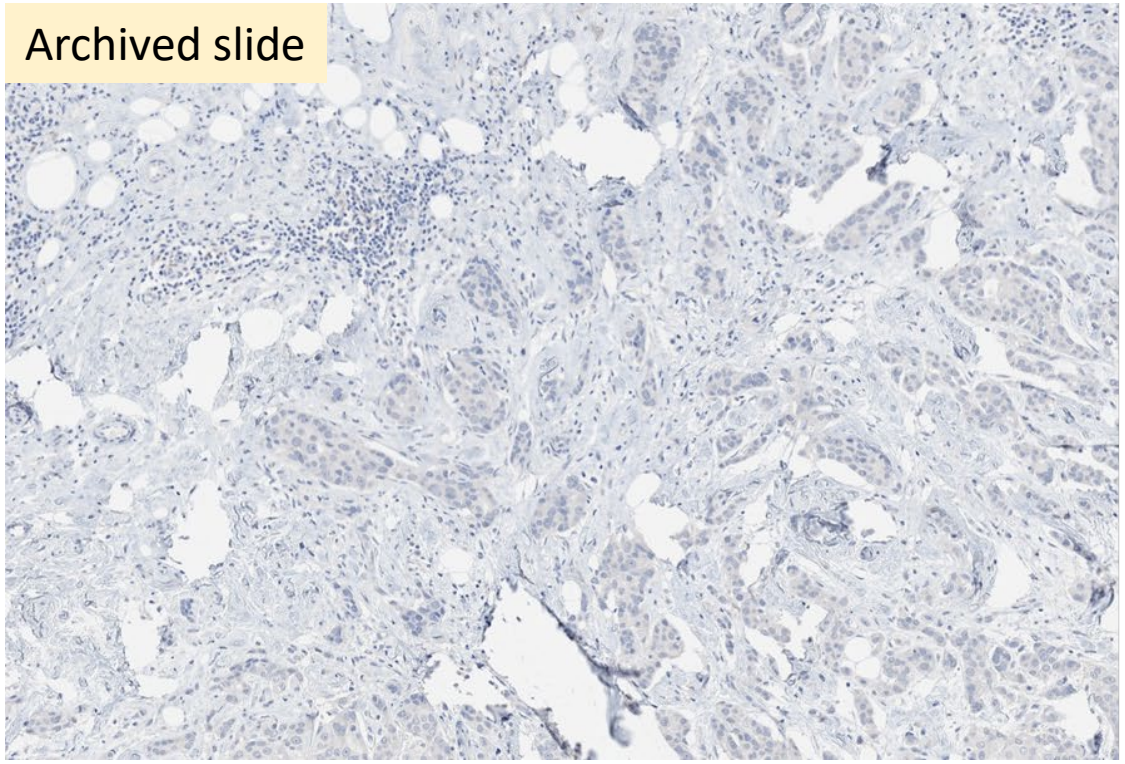
Cytology samples

Decalcified bone

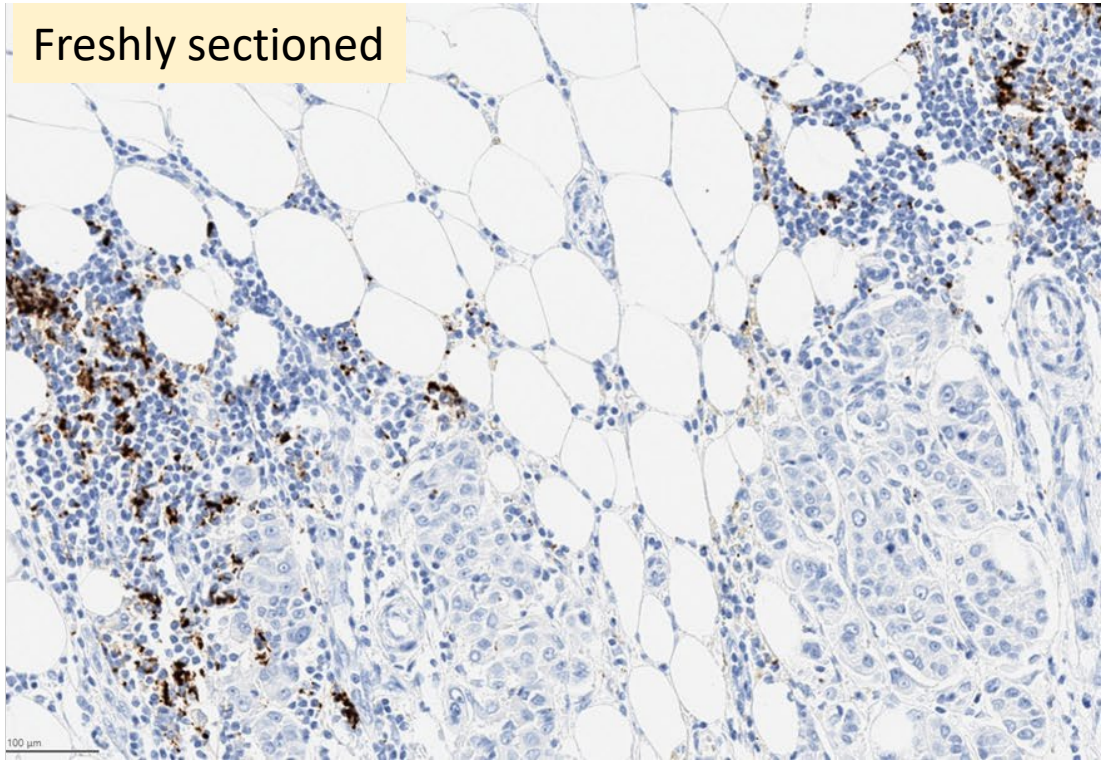
Cut slide stability

- The intensity of the staining decreased when slides were stored at room temperature past the recommended storage time

Archived slide



Freshly sectioned



PD-L1 status by sample site

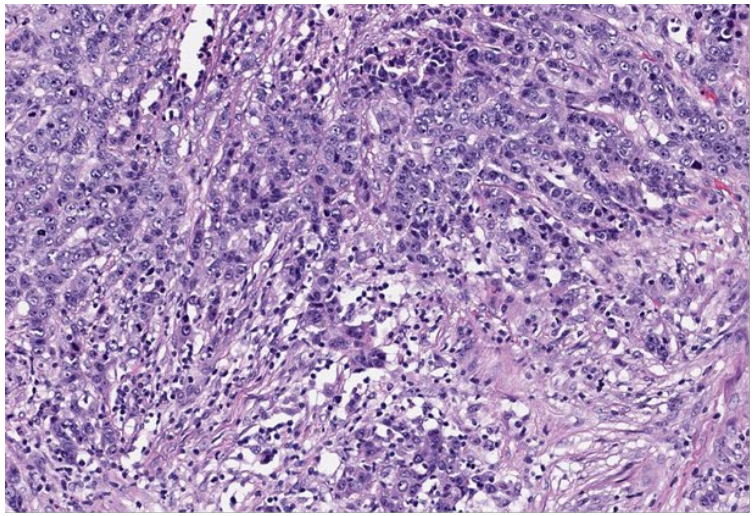
- PD-L1 SP142 IC+: 40.8% of patients in IMpassion 130 study
- Sample site: primary tumors 62.6%; metastases 37.4%
- The PD-L1 IC+ prevalence was higher in primary tumor samples (44.0%) than in metastasis (35.6%; *P* = .01)
- Liver metastasis has the lowest prevalence of PD-L1 IC+
- In matched primary and metastatic samples collected at different time points
    - PD-L1 IC+ status was concordant in 54.1% of cases

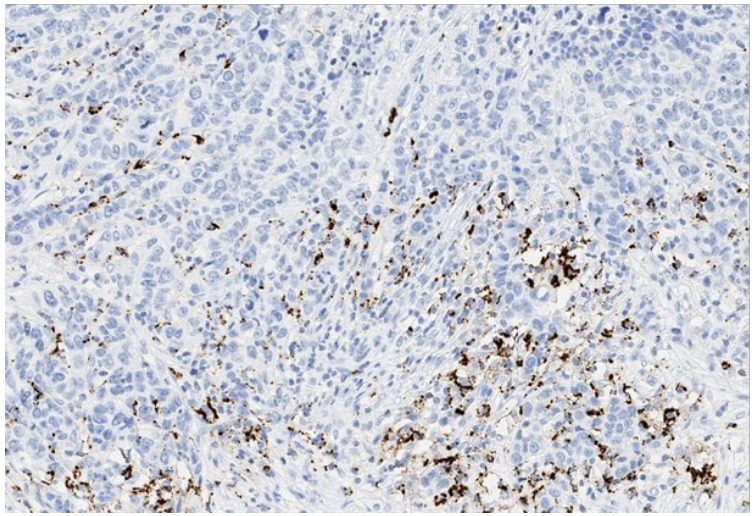
Emens LA et al. *J Natl Cancer Inst*. 2021; 113:1005-1016

Primary vs metastasis

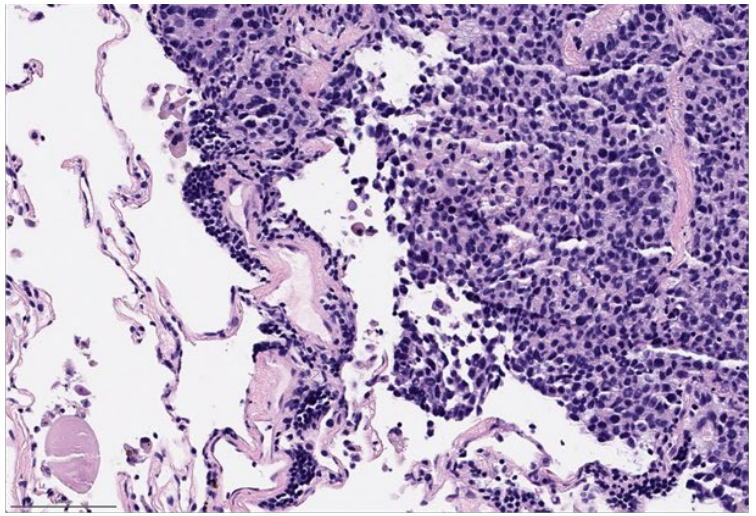
PD-L1 concordant

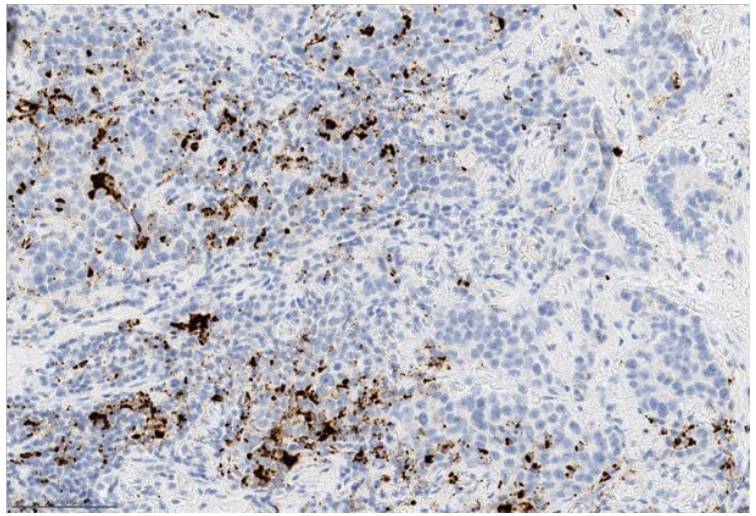
TNBC breast primary





Lung metastasis

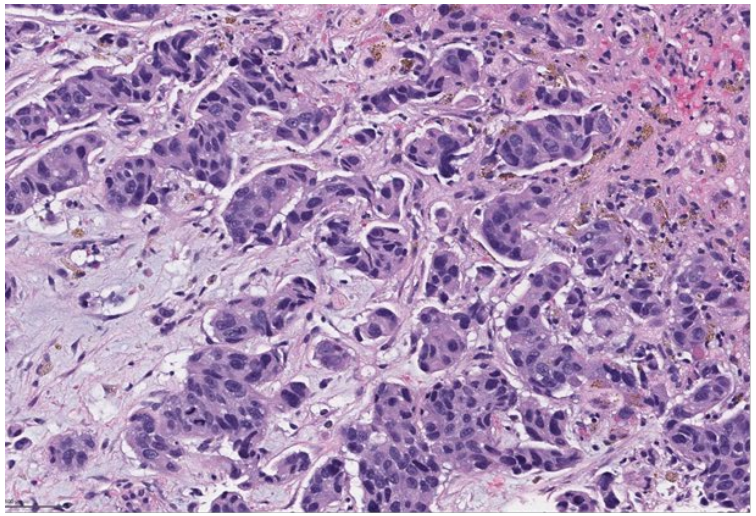


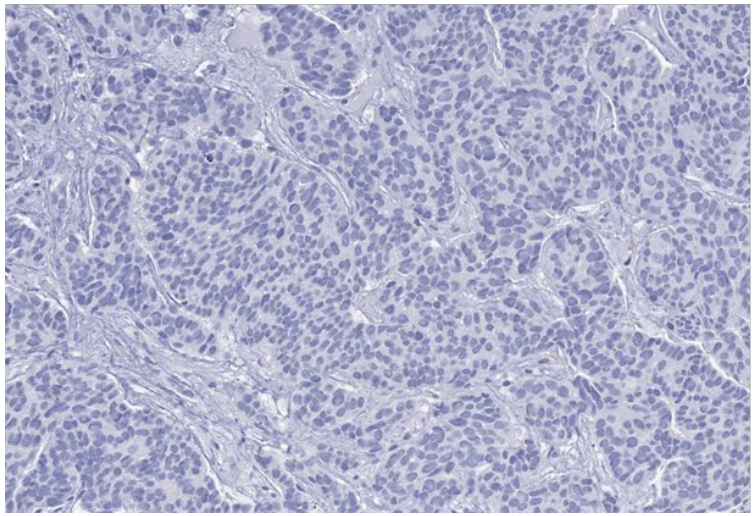


Primary vs metastasis:

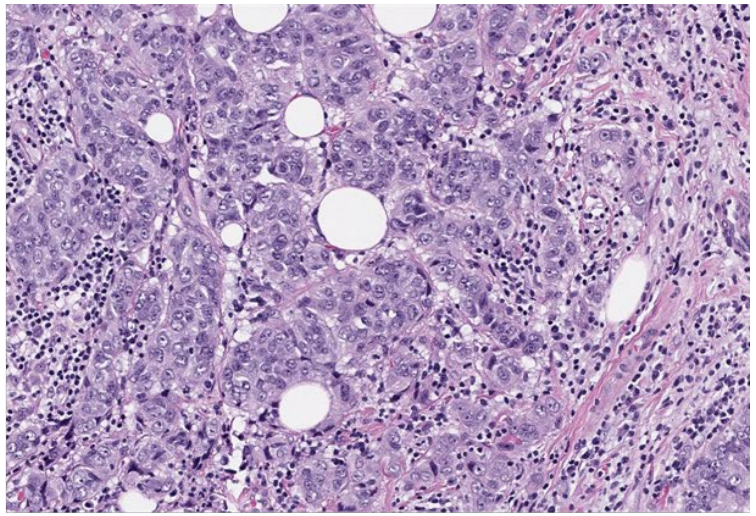
PD-L1 discordant

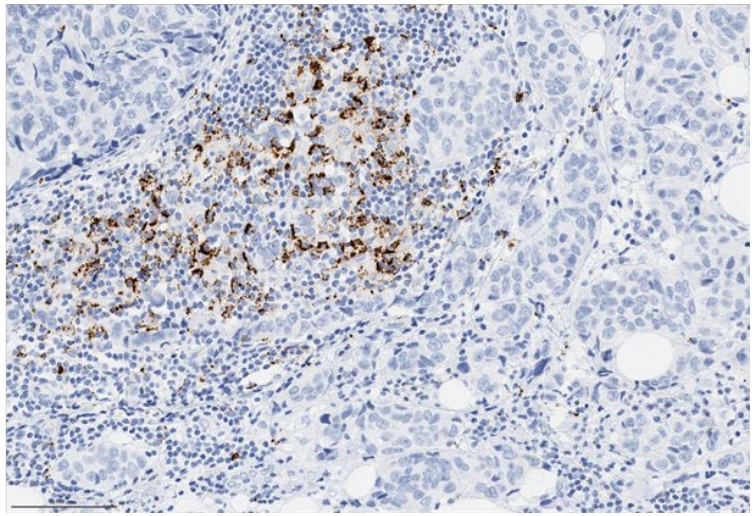
Liver metastasis





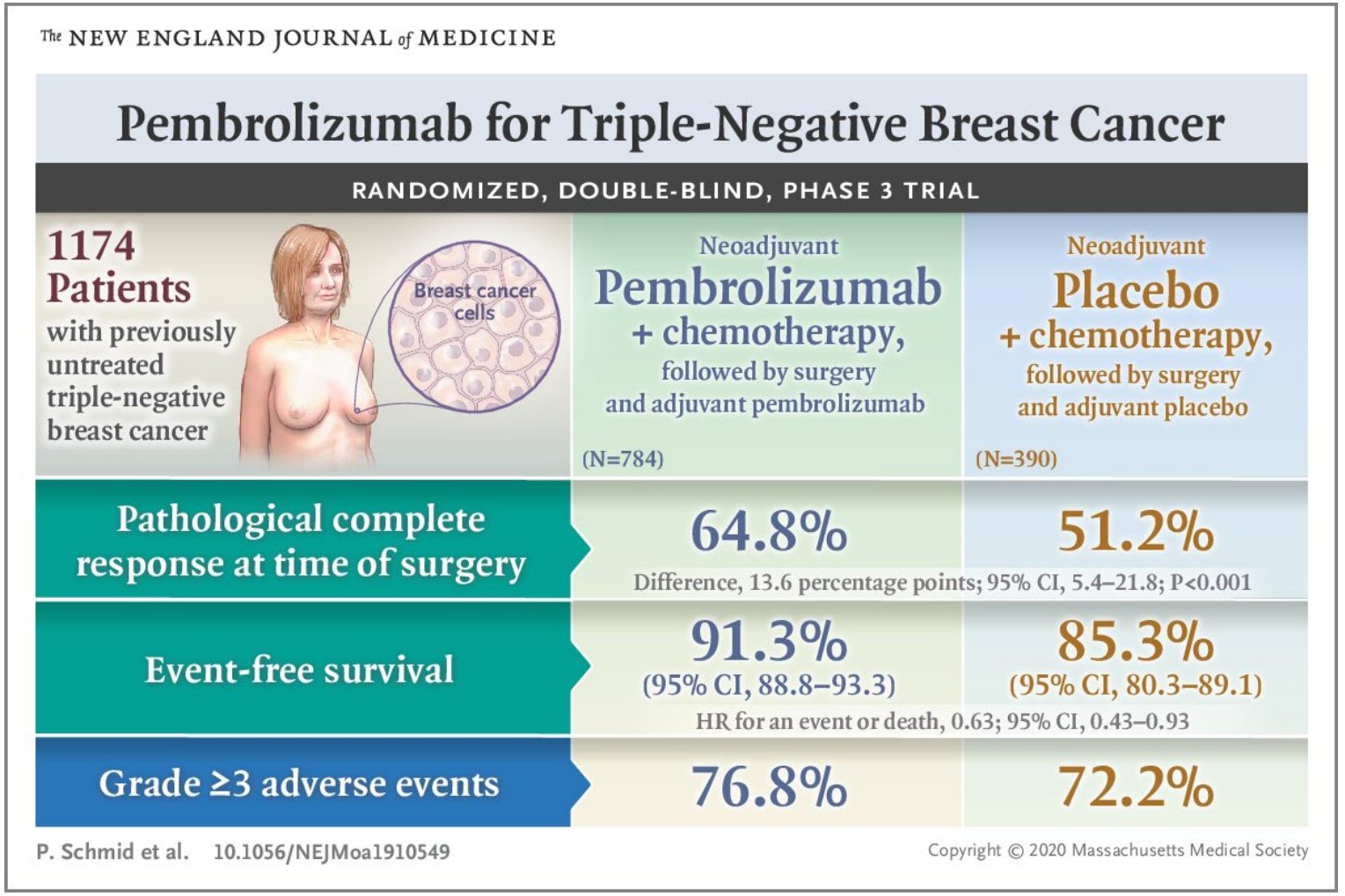
TNBC breast primary





Pembrolizumab as ***neoadjuvant*** treatment for early-stage triple negative breast cancer

In both PD-L1 positive and PD-L1 negative TNBC



Schmid P. et al. N Engl J Med. 2020; 382:810-821

Summary

Summary

HER2

- HER2 antibody-drug conjugate targeting HER2-low breast cancer
- The spectrum of HER2 IHC: HER2 negative (0), HER2-low, HER2-overexpression

Ki67

- International Ki67 in breast cancer updated recommendations: ≤ 5% vs ≥30%
- Ki67 as companion diagnostic for CDK4/6 inhibitors: the FDA removed Ki67 testing requirement

Multigene assays

- 21-gene assay: Early stage N0-N1 HR+/HER2- breast cancer

PD-L1

- Companion diagnostic for Pembrolizumab for locally advance or metastatic TNBC: CPS ≥10

Thank you