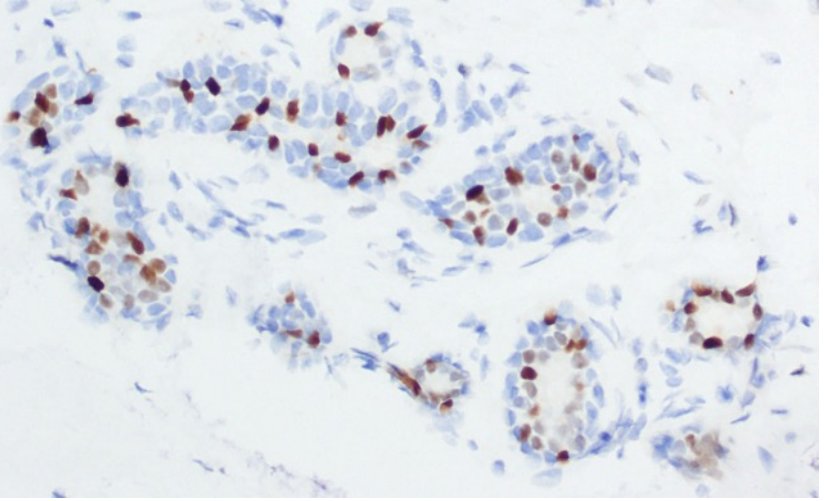
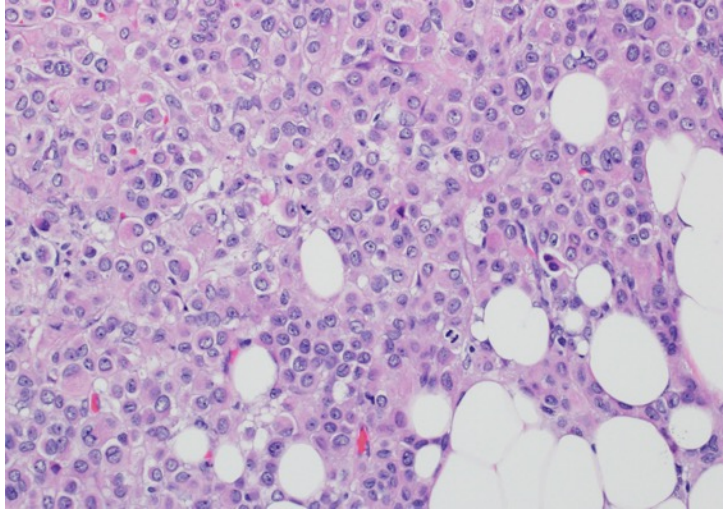




2020 Update on ER and PR Testing in Breast Cancer

Megan Troxell, MD/PhD

Stanford MEDICINE | Pathology

ISIMm
International Society for IHC and Molecular Morphology

Objectives

- Recognize the role of ER as both a prognostic and predictive marker
- Describe the criteria and clinical implications for the newly proposed ER ‘low positive’ category
- Apply and evaluate recommended external and internal controls
- Recognize discordant ER and PR as informed by breast cancer morphology

Prognostic & Predictive markers in breast cancer

Feature	Prognostic: general outcome	Predictive: response to specific therapy
Estrogen receptor (ER)	ER+ tumors less aggressive	ER+ tumors respond to anti-hormonal therapy
HER2	HER2+ tumors more aggressive	HER2+ tumors respond to anti-HER2 Rx
Recurrence score (if ER+)	Low recurrence score less aggressive	Low recurrence score less benefit from cytotoxic chemotherapy

ER: The first predictive marker

Molecular era

Ligand Binding Assays (DCC) 1970's

ELISA 1980's

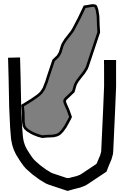
Immunohistochemistry 1990's→ present

IHC-image analysis (AI?)

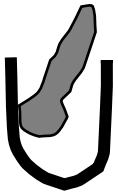
RT-PCR ?

Multigene assays

Other ?



Fresh



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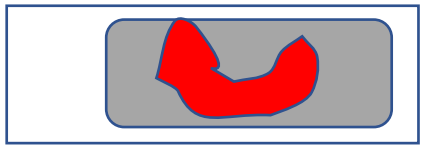
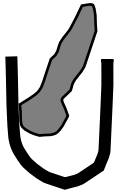


FFPE

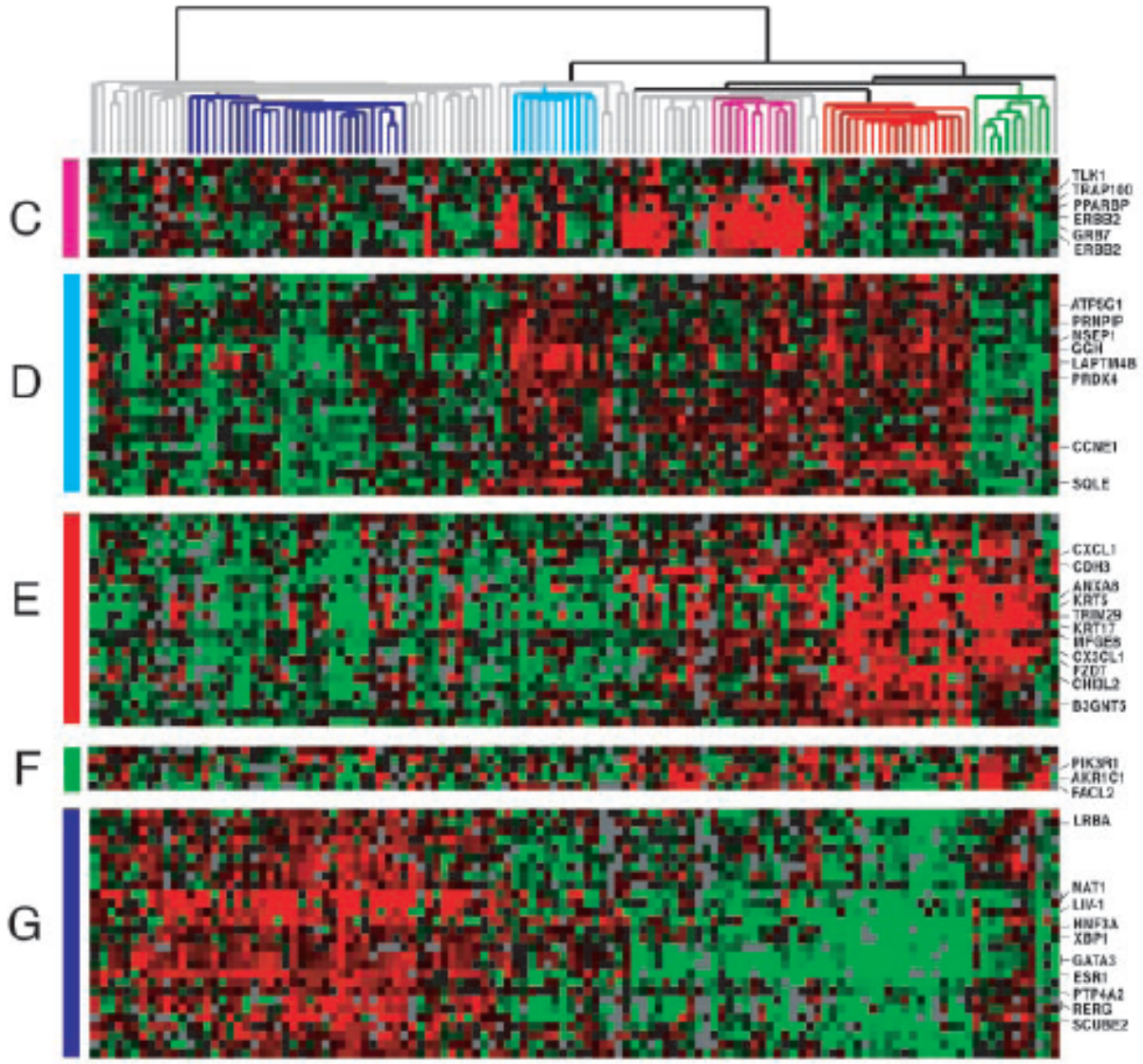


FFPE

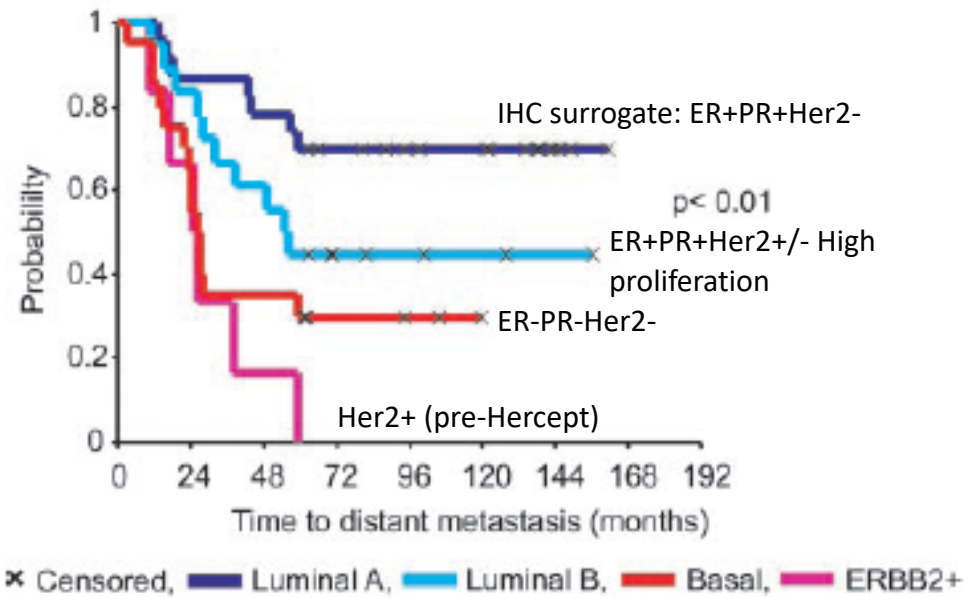
"No other assay types are recommended as the primary screening test for ....predicting benefit from endocrine therapy"

Gene Expression Profiling

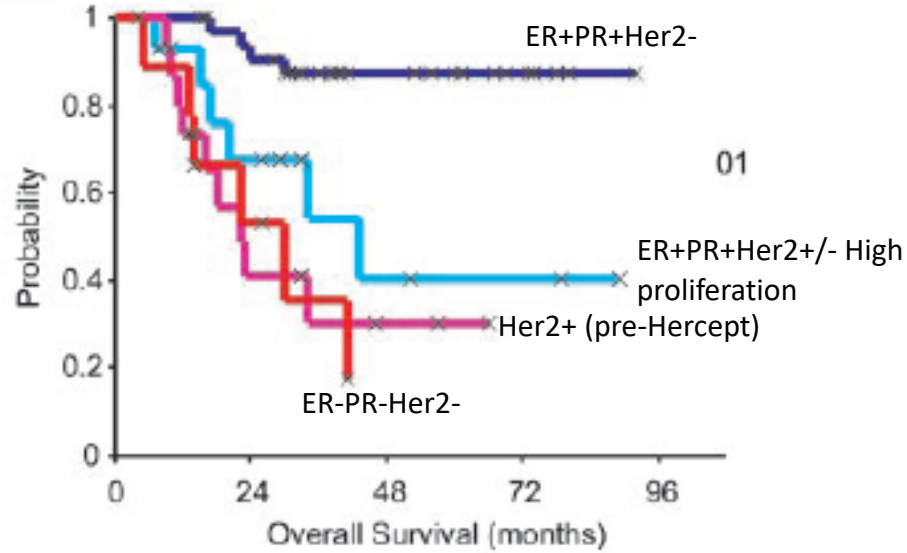
Molecular (intrinsic) subtypes: luminal, HER2, basal



A van't Veer data set Sorlie. PNAS. 100:8418-23.

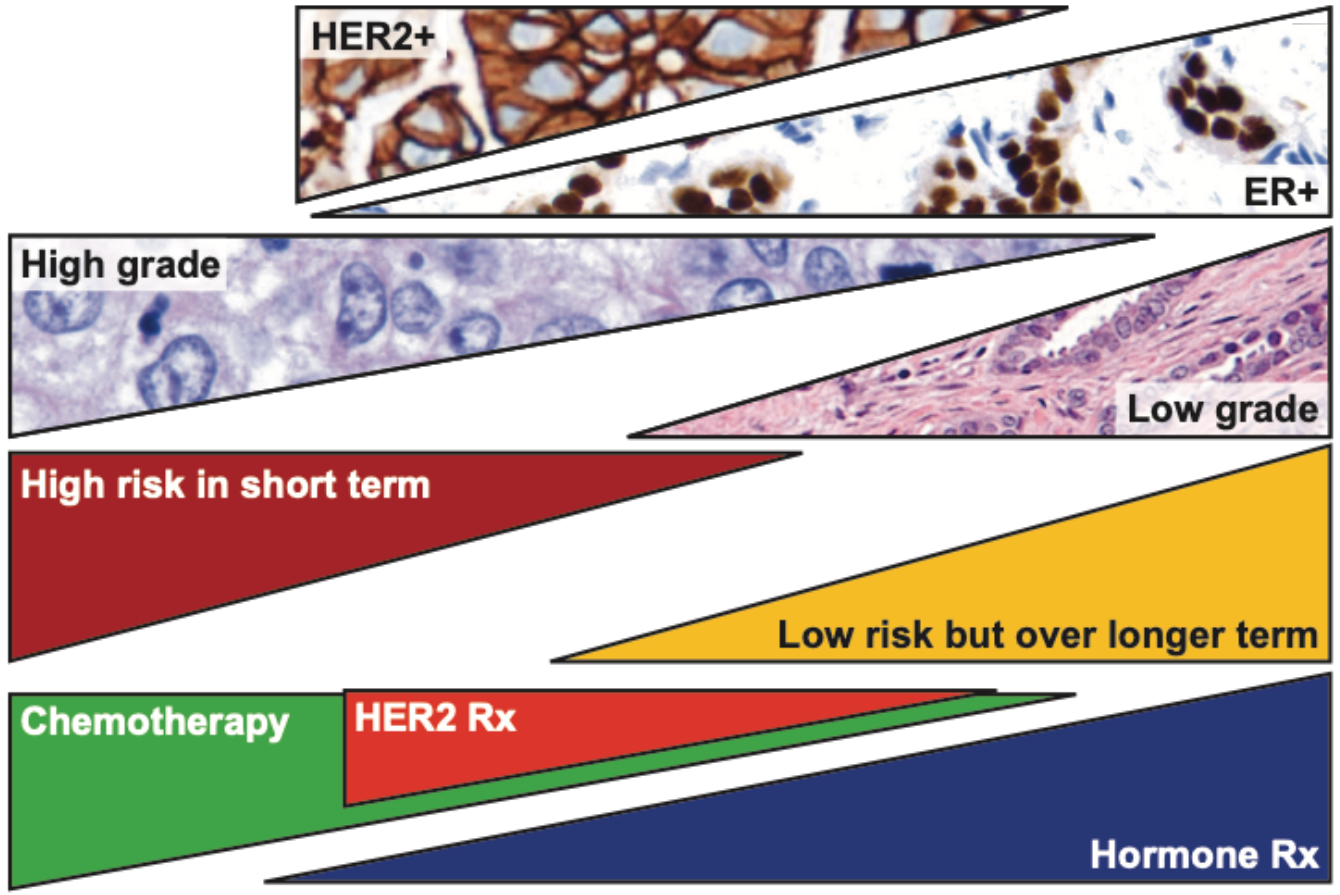


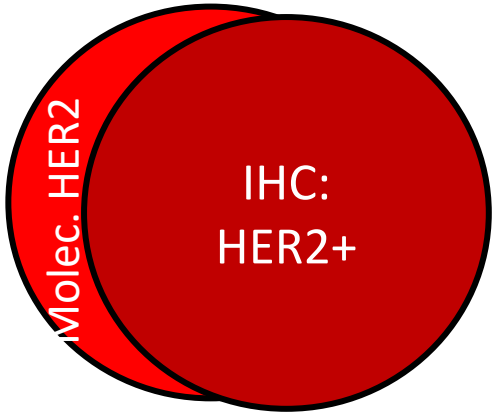
B Norway/Stanford data set

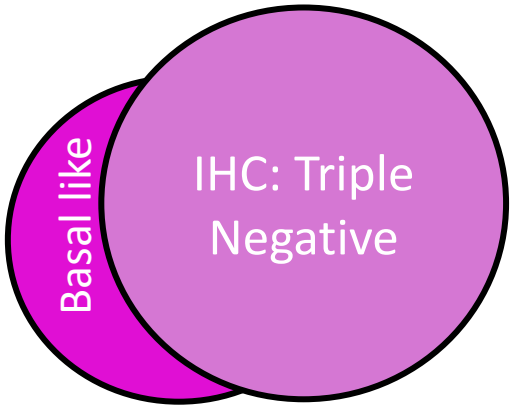


Correlation of Breast Cancer Molecular Subtypes with Clinicopathologic Features

Molecular Subtypes:	Basal	HER2-E	Luminal B	Luminal A
% of breast cancers:	15-20%	10-20%	20-30%	40-60%
Receptor expression:				
Histologic grade:				
Recurrence risk:				
Therapies used:				







TNBC: other subclasses

Allison KH. WHO 5th ed. Fig 2.83

Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma

Tang & Tse. Arch Pathol Lab Med. 2016;140:806–14; WHO 5th edition

	Luminal A-like	Luminal B-like	Luminal HER2+	HER2+	TN-Basal	TN-other
ER	+	+	+	-	-	-
PR	+	+/low/-	+	-	-	-
HER2	-	-	+	+	-	-
Ki-67	Low*	High*	Any	Any	Often high	-
CK5/ER					+	-

*Ki-67 cut point varies between 14% and 20% in St. Gallen criteria & WHO
Luminal B: low PR or high Ki-67

Special Types of Breast Cancer

Type	Rate	Hormones
Lobular, classic	5-15%	**ER+** Her2-
Tubular, pure	<2%	**ER+** Her2-
Cribriform, pure	0.8-3.5%	**ER+** Her2-
Mucinous, pure	~2%	**ER+** Her2-
Neuroendocrine	2-5%	ER+ Her2-
Micropapillary	<2%	ER+ Her2+/-
Apocrine	<4%	ER- Her2- AR+ (TN*)
Adenoid cystic	0.1%	ER- Her2- (TN*)
Secretory	<0.15%	ER- Her2- (TN*)
Metaplastic	<1%	ER- Her2- (TN)

Weigelt & Reis-Filho Nat Rev Clin Oncol 2009; 6:718-30.

Low grade IDC/ILC should be ER+

*Some special types of TN less aggressive

Nadji. AJCP2005;123:21-27

Type of Carcinoma	ER+	PR+
Infiltrating ductal, not otherwise specified (n = 4,396)	3,255 (74)	2,330 (53)
Tubular (n = 237)	237 (100)	225 (95)
Colloid (n = 184)	184 (100)	133 (72)
Papillary (n = 44)	44 (100)	35 (80)
Apocrine (n = 40)	0 (0)	0 (0)
Medullary (n = 96)	0 (0)	0 (0)
Metaplastic (n = 120)	0 (0)	0 (0)
Infiltrating lobular (n = 380)	380 (100)	293 (77)

Estrogen and Progesterone Receptor Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Guideline Update

Arch Pathol Lab Med. 2020;144:545-563; *JCO* 2020;38:1346-66

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; Antonio C. Wolff, MD[21]

THE BOTTOM LINE—Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update

Guideline Questions

1. What are the optimum quality assurance (QA), tissue handling, scoring system, and reporting for determining potential benefit from endocrine therapy?
2. What additional strategies can promote optimal performance, interpretation, and reporting of immunohistochemistry (IHC) assays, particularly in cases with low estrogen receptor (ER) expression?
3. Are other ER expression assays acceptable for identifying patients likely to benefit from endocrine therapy?
4. Should ductal carcinoma in situ (DCIS) be routinely tested for hormone receptors?

Estrogen and Progesterone Receptor Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Guideline Update

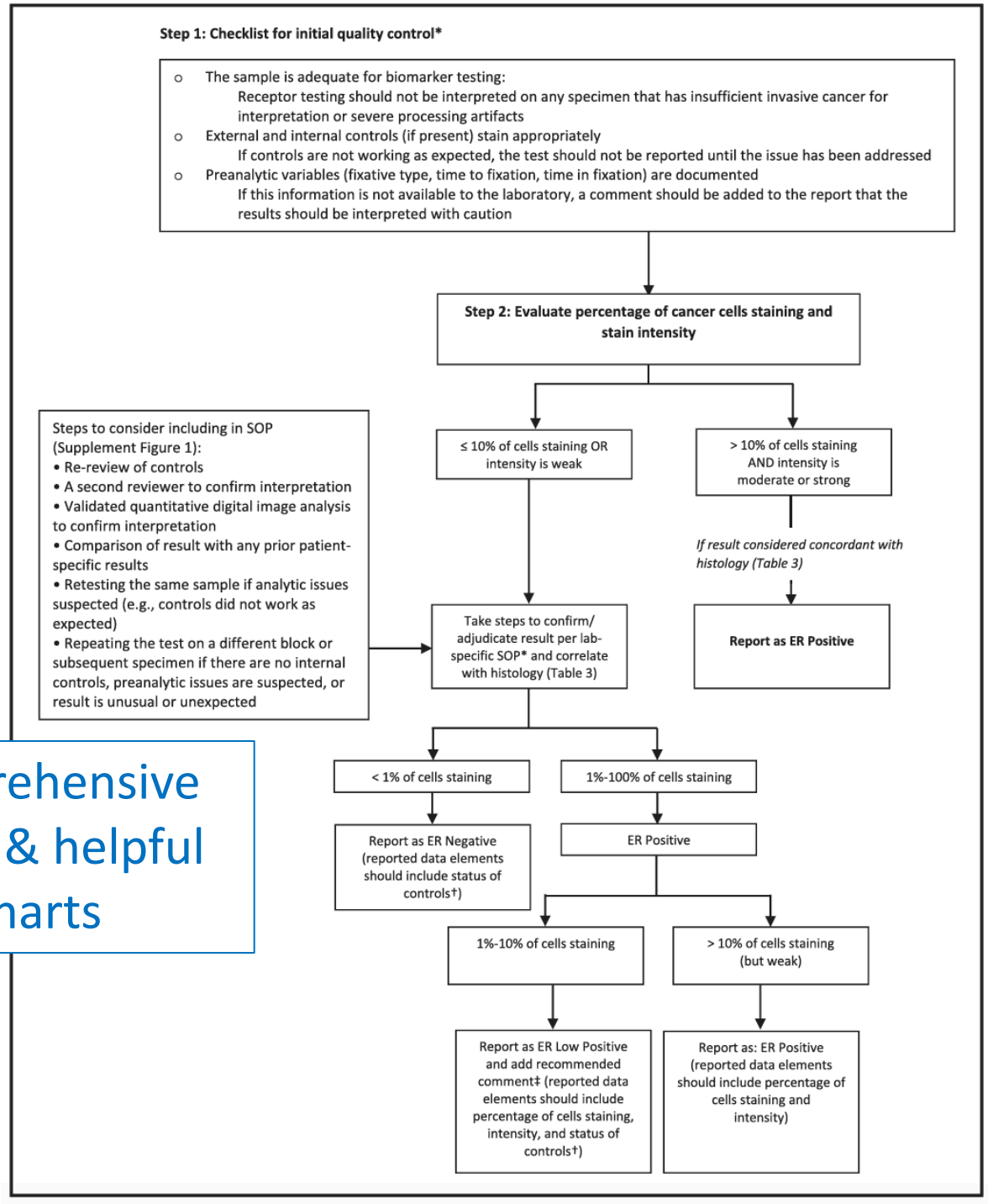
Arch Pathol Lab Med. 2020;144:545-563; *JCO* 2020;38:1346-66

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; Antonio C. Wolff, MD[21]

Table 1. Summary of All Recommendations

2010 Recommendation	Updated Recommendation
Clinical Question 1. What are the optimum QA, specimen handling, positive threshold, scoring system, and reporting for determining potential benefit from endocrine therapy?	
Optimal algorithm for ER/PgR testing	**Optimal algorithm for ER/PgR testing**
Positive for ER or PgR if finding that ≥ 1% of tumor cell nuclei are immunoreactive.	Samples with 1%–100% of tumor nuclei positive for ER or PgR are interpreted as positive.
Negative for ER or PgR if finding that < 1% of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls are seen).	For reporting of ER (not PgR), if 1%–10% of tumor cell nuclei are immunoreactive, the sample should be reported as ER Low Positive with a recommended comment (Table 2; Figure 1).
Uninterpretable for ER or PgR if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining.	A sample is considered negative for ER or PgR if < 1% or 0% of tumor cell nuclei are immunoreactive.
	A sample may be deemed uninterpretable for ER or PgR if the sample is inadequate (insufficient cancer or severe artifacts present, as determined at the discretion of the pathologist), if external and internal controls (if present) do not stain appropriately, or if preanalytic variables have interfered with the assay's accuracy (Figures 1 to 4).
	Clinicians should be aware of and be able to discuss with patients the limited data on ER–low positive cases and issues with test results that close to a positive threshold.
Optimal testing conditions	**Optimal testing conditions (no changes)**
Large (preferably multiple) core biopsies of tumor are preferred for testing if they are representative of the tumor (grade and type) at resection.	Large (preferably multiple) core biopsies of tumor are preferred for testi they are representative of the tumor (grade and type) at resection.
Accession slip and report must include guideline-detailed elements.	Accession slip and report must include guideline-detailed elements.
Optimal tissue handling requirements	**Optimal tissue handling requirements (no changes)**
Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PgR testing are fixed in 10% NBF for 6 to 72 hours. Samples should be sliced at 5-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of NBF to allow adequate tissue penetration. If tumor comes from remote location, it should be bisected through the tumor on removal and sent to the laboratory immersed in a sufficient volume of NBF. Cold ischemia time, fixative type, and time the sample was placed in NBF must be recorded.	Time from tissue acquisition to fixation should be as short as possible. Samples for ER and PgR testing are fixed in 10% NBF for 6 to 72 hours. Samples should be sliced at 5-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of NBF to allow adequate tissue penetration. If tumor comes from remote location, it should be bisected through the tumor on removal and sent to the laboratory immersed in a sufficient volume of NBF. Cold ischemia time, fixative type, and time the sample was placed in NBF must be recorded.
As in the ASCO/CAP HER2 guideline, use of slides cut more than 6 weeks before analysis is not recommended.	As in the ASCO/CAP HER2 guideline, use of unstained slides cut more than 6 weeks before analysis is not recommended.

Comprehensive tables & helpful flow charts



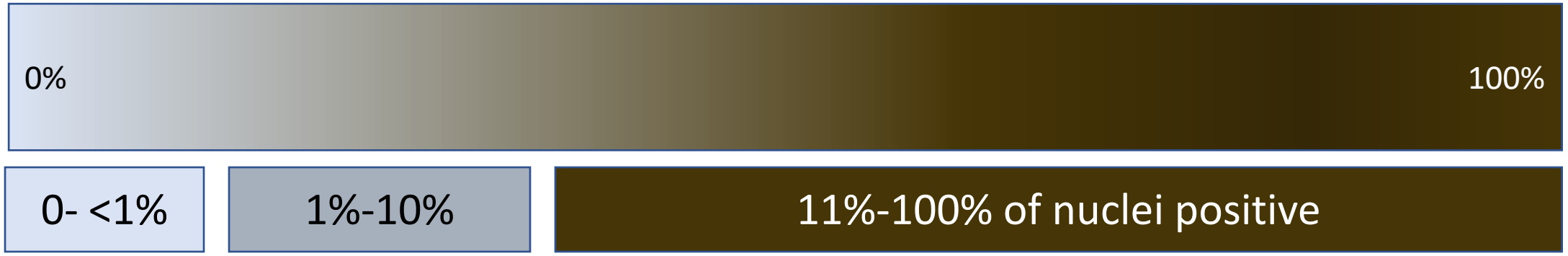
Appropriate ER threshold?

- Focus on ER Low positive
  - 1-10% of tumor cell nuclei immunoreactive

- Biologic low mRNA/protein expression?
- Erroneously low ER results in a truly ER-positive tumor?
- Borderline (false) positive IHC results in an ER negative tumor?
  - Test reproducibility?
- Small ER+ subpopulation

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

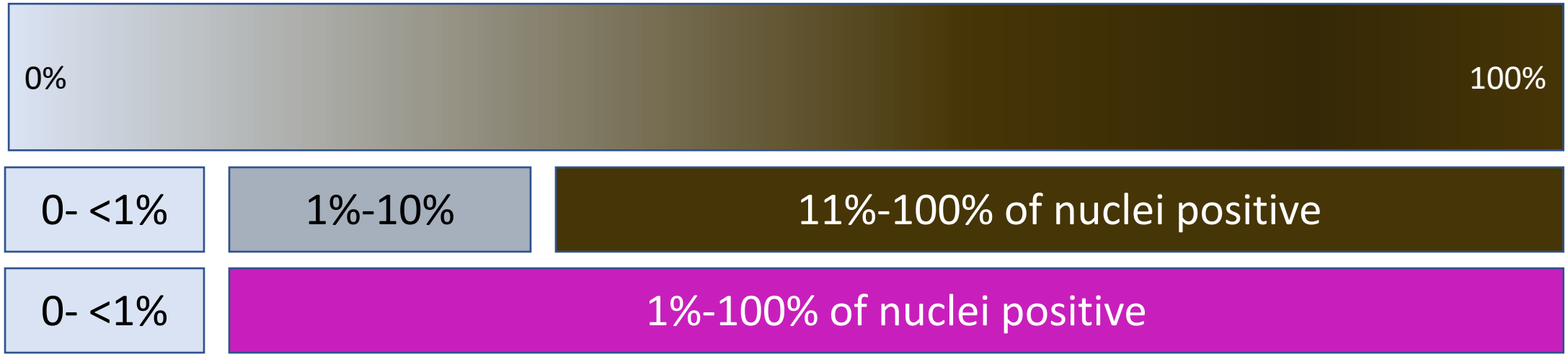
Focus on ER Low positive: 1-10% of tumor cell nuclei immunoreactive



Biologic continuum with arbitrary cut points

After Allison KH unpublished

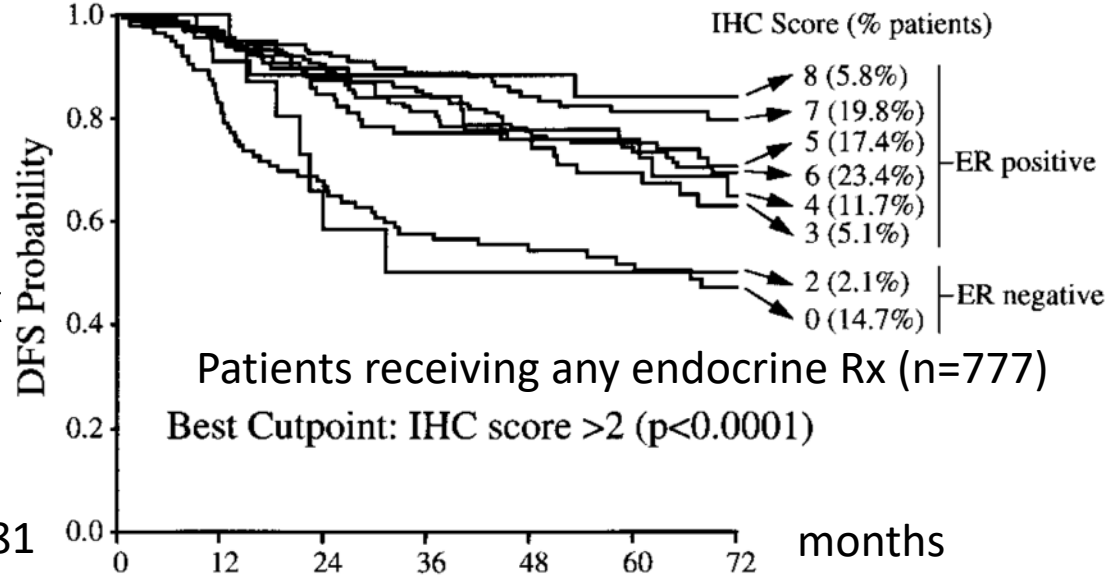
Focus on ER Low positive: 1-10% of tumor cell nuclei immunoreactive



To select high likelihood of benefit from Endocrine Rx
→use 1%

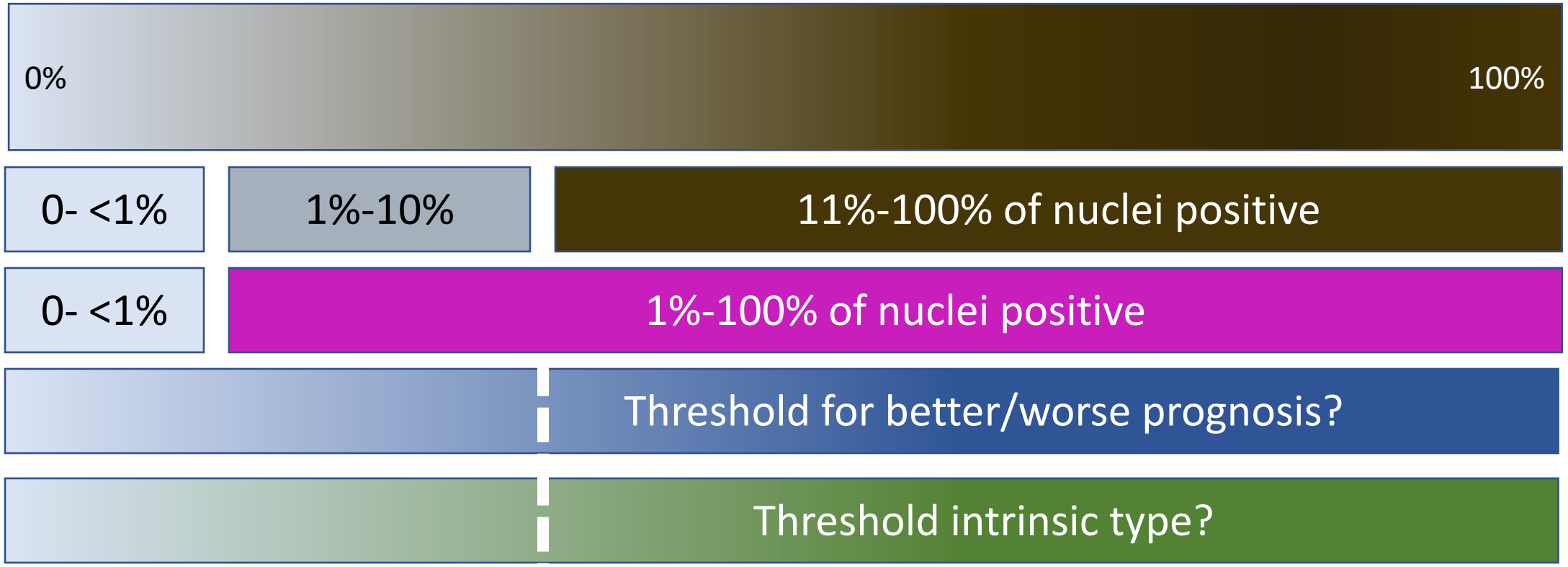
To select those who will not benefit from Endocrine Rx
→use <1%

After Allison KH unpublished



Harvey…Allred.
1999;17:1474-81

Focus on ER Low positive: 1-10% of tumor cell nuclei immunoreactive



To select overall treatment pathway →use 10%

Triple negative trials, neoadjuvant chemotherapy

To forecast overall prognostic group →use 10%

After Allison KH unpublished

ER Low Positive: 1-10% of tumor cell nuclei immunoreactive

- May benefit from hormonal therapy
- BUT, heterogeneous group
  - “clinical outcomes and biologic/molecular profiles that are often more similar to those of ER-negative cancers”
  - “base decisions on the totality of information available"

Intrinsic type	ER 0 (n=183)	ER 1-9% (n=25)	ER>10% (n=251)
Luminal A	1%	**0%**	48%
Luminal B	<1%	**8%**	24%
Basal	61%	**48%**	6%

ER Low Positive should not disqualify appropriate patients from TNBC trials or therapy

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Iwamoto. J Clin Oncol 30:729-34

ER Low positive: 1-10% tumor nuclei immunoreactive

Recommended comment:

“The cancer in this sample has a low level (1%–10%) of ER expression by IHC. There are limited data on the overall benefit of endocrine therapies for patients with low level (1%–10%) ER expression, but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results are heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER-negative cancers.”

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

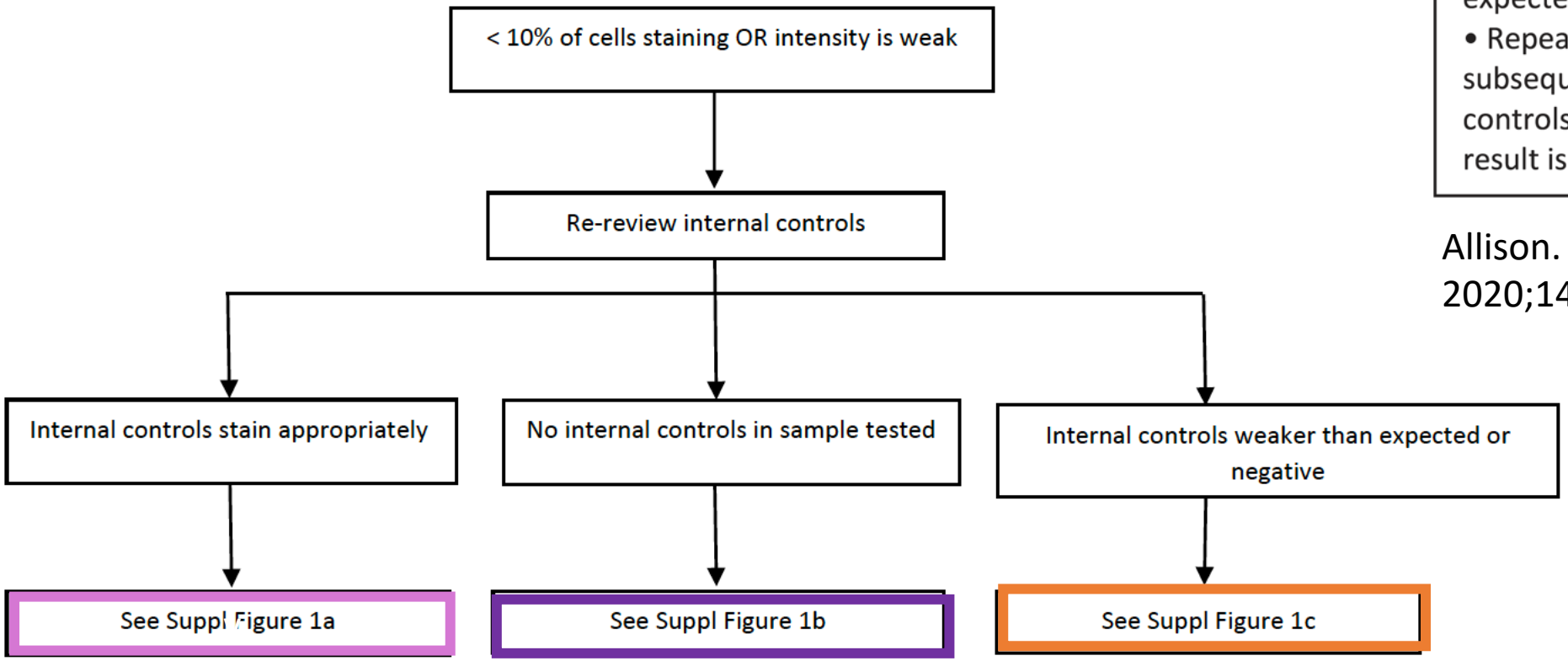
ER Low positive: 1-10% tumor nuclei immunoreactive

- Reproducibility
- Laboratories should **establish and follow an SOP** stating the steps the laboratory takes to confirm or adjudicate ER results for cases with weak stain intensity or 10% of cells staining
- The status of internal controls should be reported for cases with 0%–10% staining. For cases with these results without internal controls present and with positive external controls, an additional report comment is recommended

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Guideline supplement: ER Weak or Low positive SOP

Data Supplement 2: Figure 1. Example of a Lab-Specific Standard Operating Procedure for cases with initial ER IHC result with < 10% of cells staining or stain intensity is weak

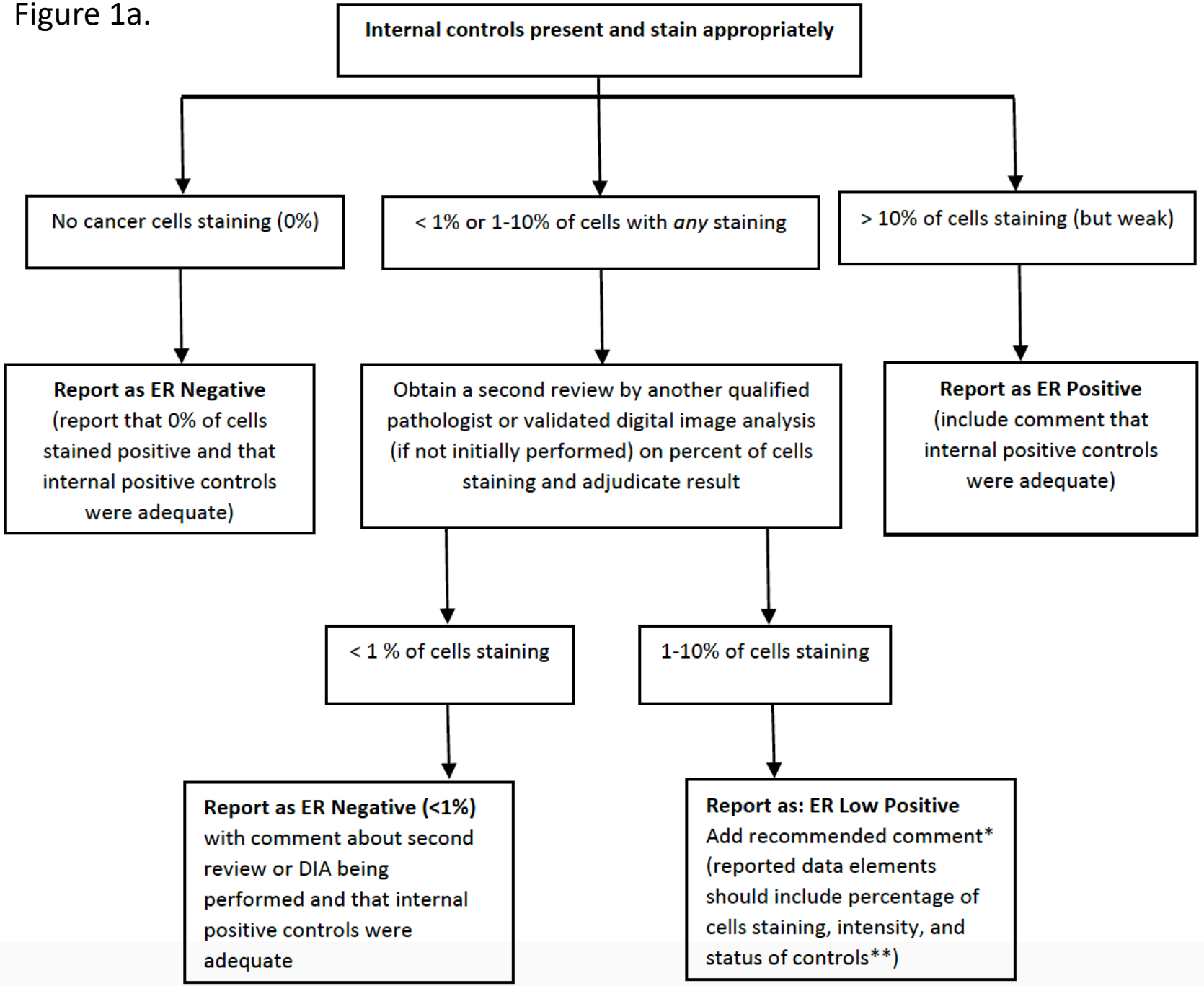


Steps to consider including in SOP (Supplement Figure 1):

- Re-review of controls
- A second reviewer to confirm interpretation
- Validated quantitative digital image analysis to confirm interpretation
- Comparison of result with any prior patient-specific results
- Retesting the same sample if analytic issues suspected (e.g., controls did not work as expected)
- Repeating the test on a different block or subsequent specimen if there are no internal controls, preanalytic issues are suspected, or result is unusual or unexpected

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Figure 1a.

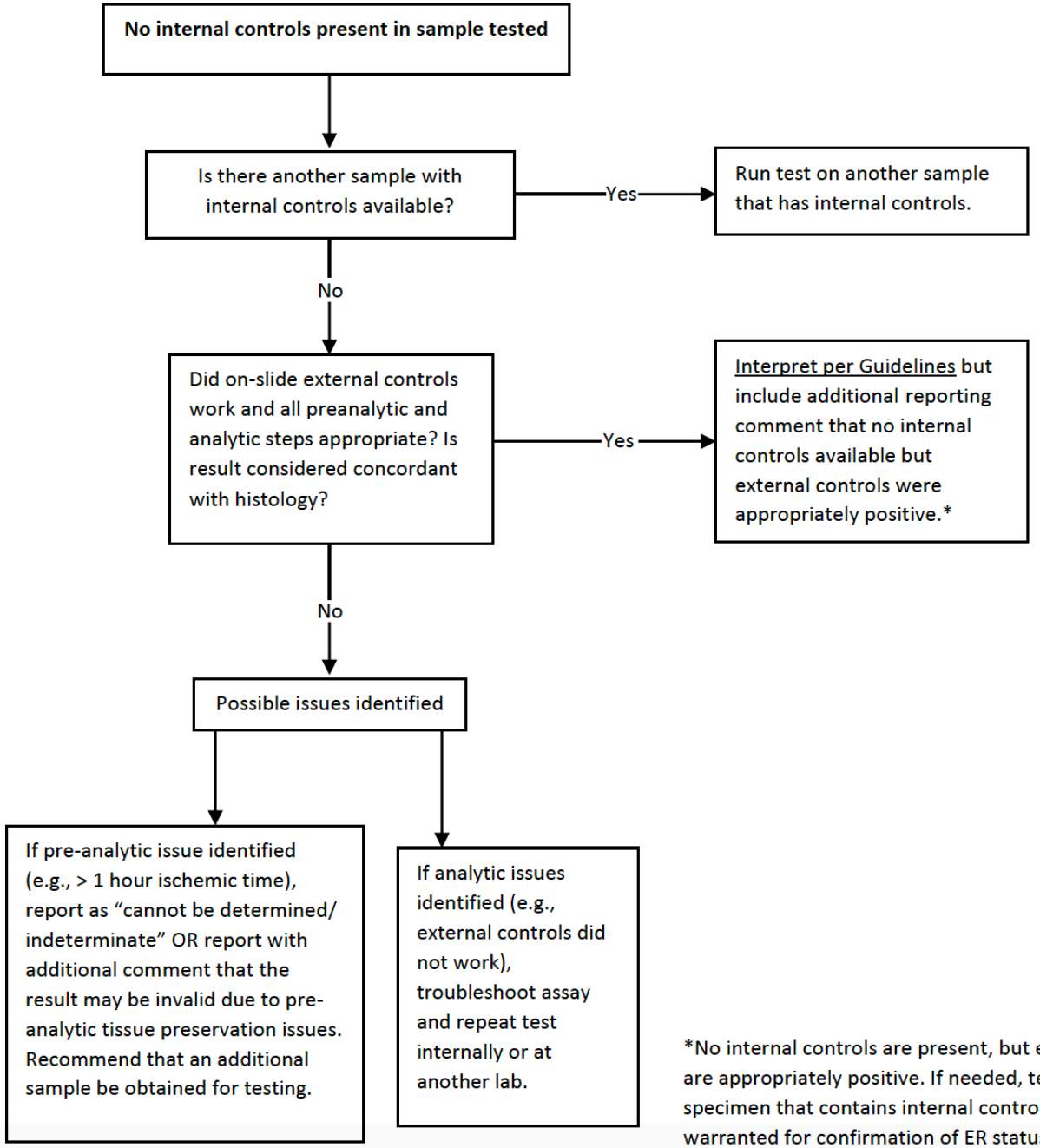


Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Guideline supplement

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

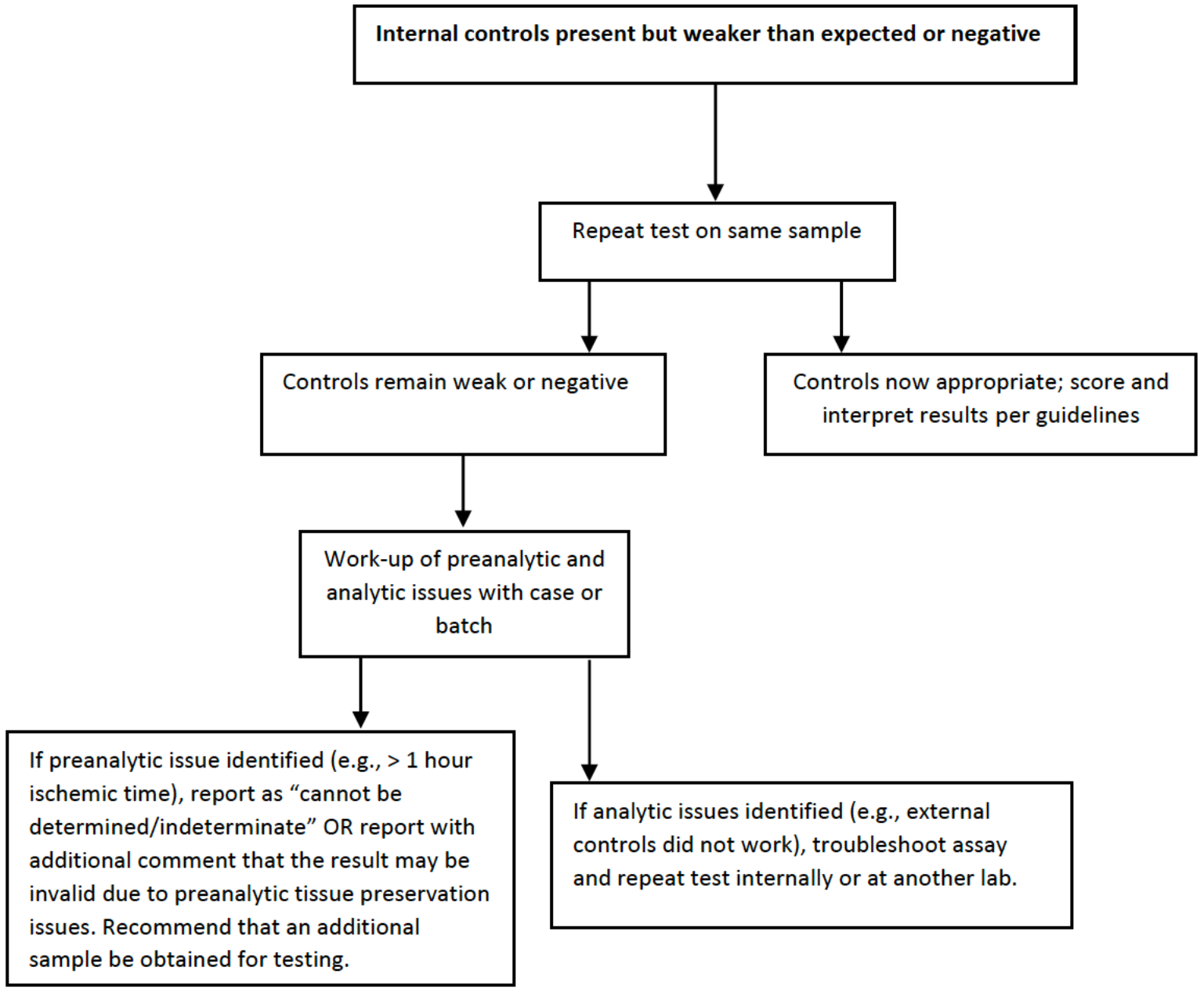
Figure 1b. No internal controls present in sample tested



Guideline supplement

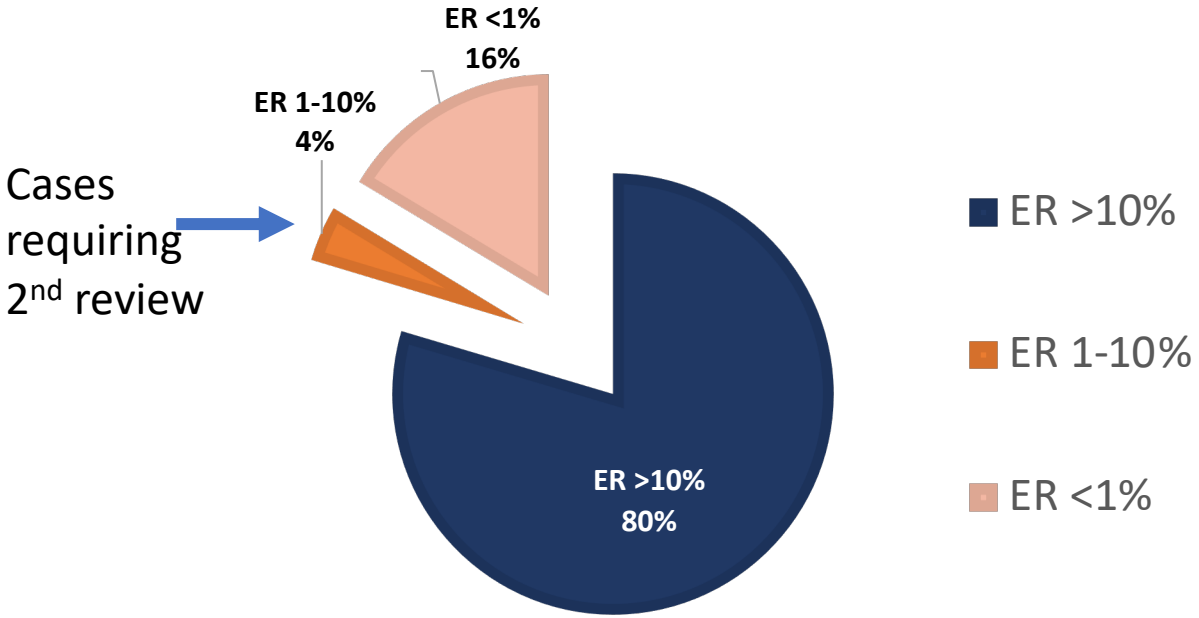
Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Figure 1c. Internal controls present but weaker than expected or negative



ER low positive: our experience

Category (Based on Majority)	# Cases	Cases with 100% (6 of 6) agreement	Cases with >80% (5 of 6) agreement
Negative (<1%)	16	67%	87%
Low Positive (1-10%)	6	0%	17%
Positive (>10%)	8	75%	100%

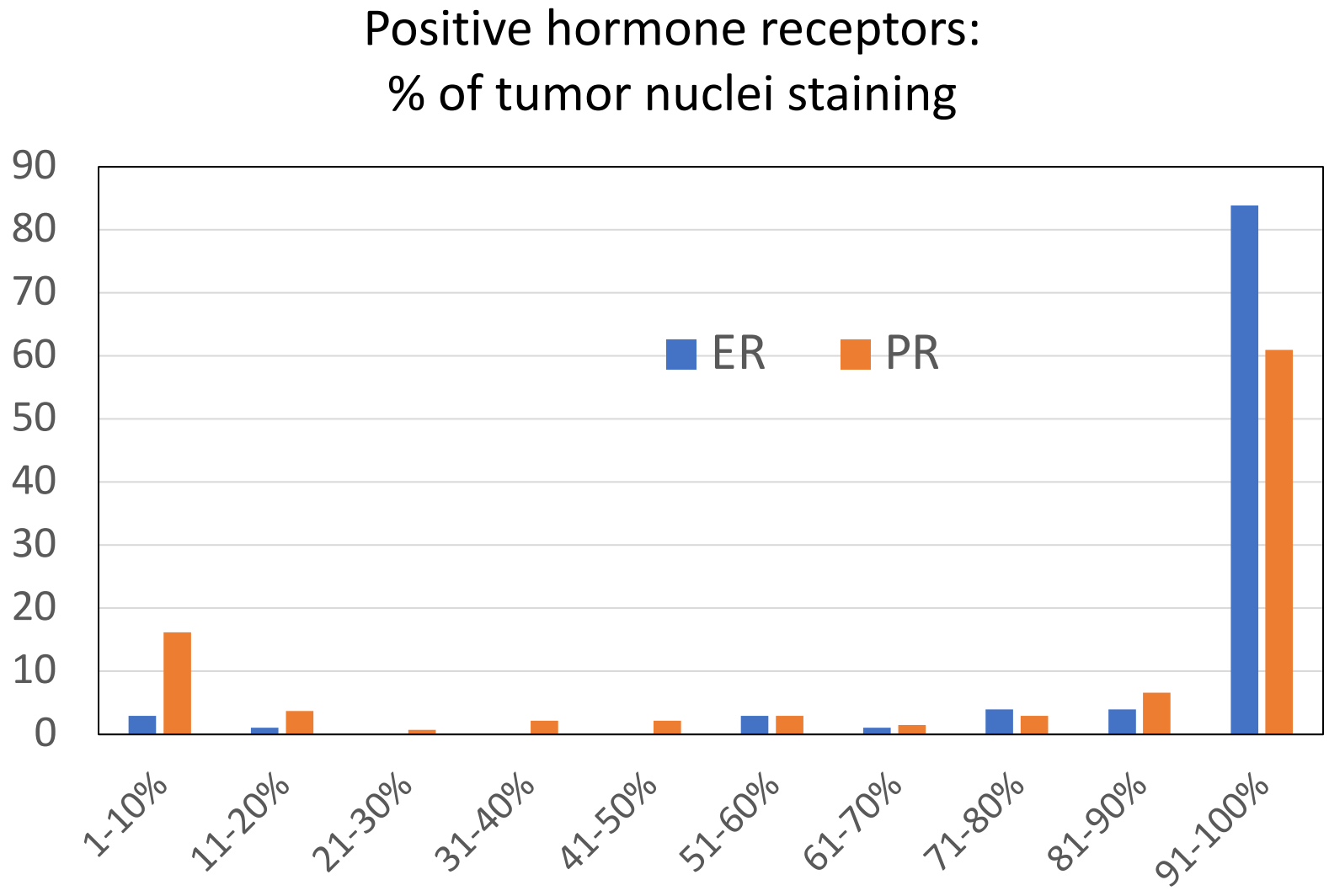


- Disagreements esp. between Negative v Low Positive results (61%)
- All disagreements close to the 1% threshold
- SOP now requires a second pathologist review:
  - Prior to reporting any case with 1-10% ER staining
  - Consider if close to <1% threshold.

Winters C, Allison KH, unpublished

CAP Q-probes

- CAP Q-probes
- N=21 labs
- 687 breast cancer cases (2019)
- Overall
  - 86% ER+
    - ER 1-10%: 3% of cases
  - 75% PR+
    - PR more heterogeneity



- Yale 1% low ER

Caruana D, et al. NPJ Breast Cancer. 2020 Feb 5;6:5.
doi: 10.1038/s41523-020-0146-2.

Pre-analytic factors

- Testing of core biopsies reaffirmed
- Ischemia/fixation parameters re-affirmed
  - Ischemia as short as possible
  - 10% NBF 6-72 hours
  - Document
- Age of cut slides >6 weeks

Further impromptu comments on:

- Rapid processing

- **Decalcification**
- **Cytology fixative (alcohol based)**
  - **Widely variable between labs**
  - **Very important for correct therapy of metastatic disease**

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

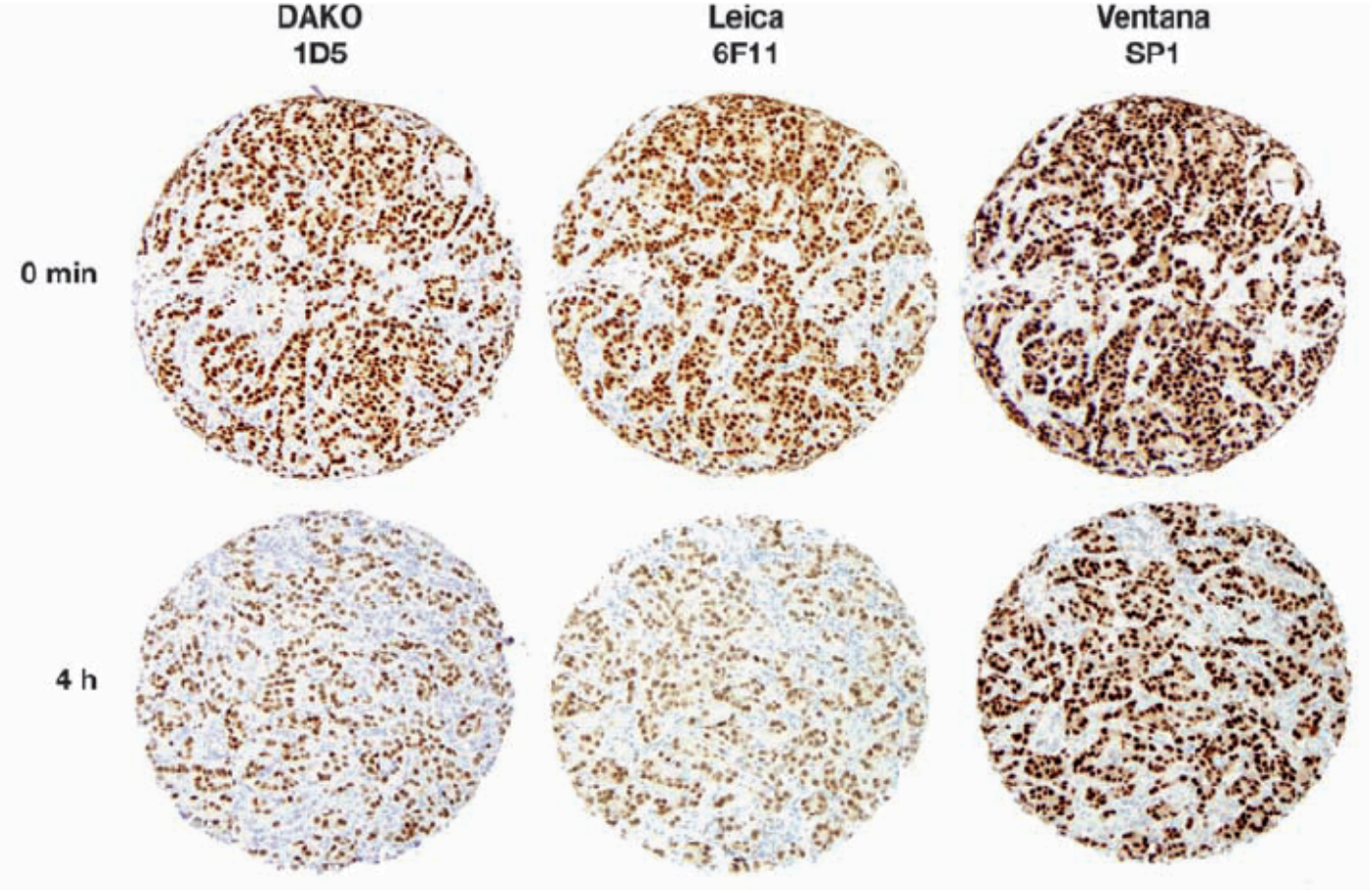
Effect of Delayed Formalin Fixation on Estrogen and Progesterone Receptors in Breast Cancer

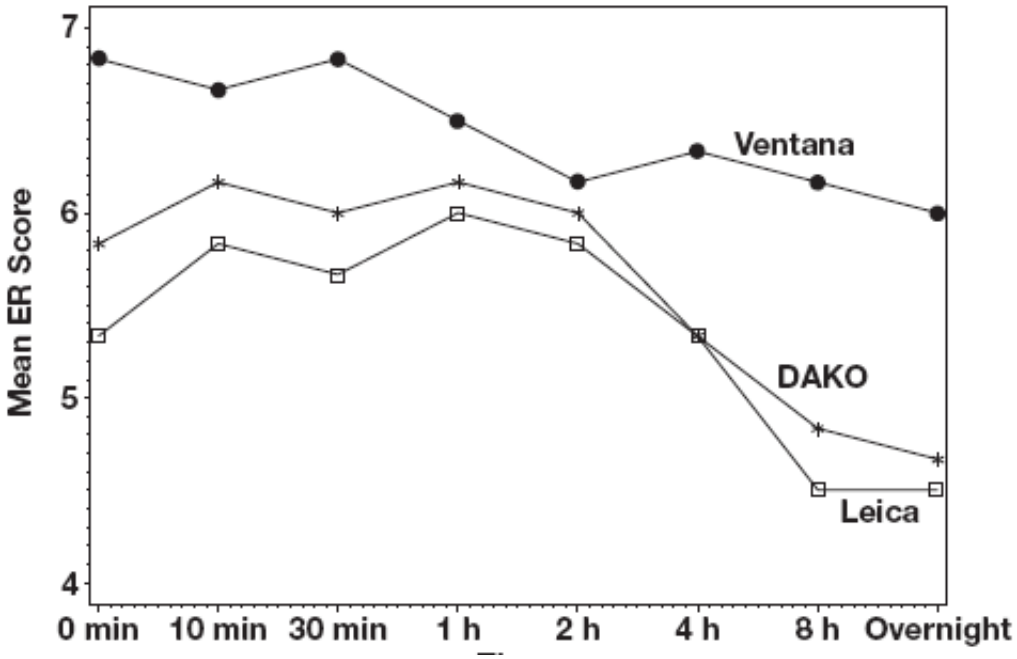
A Study of Three Different Clones

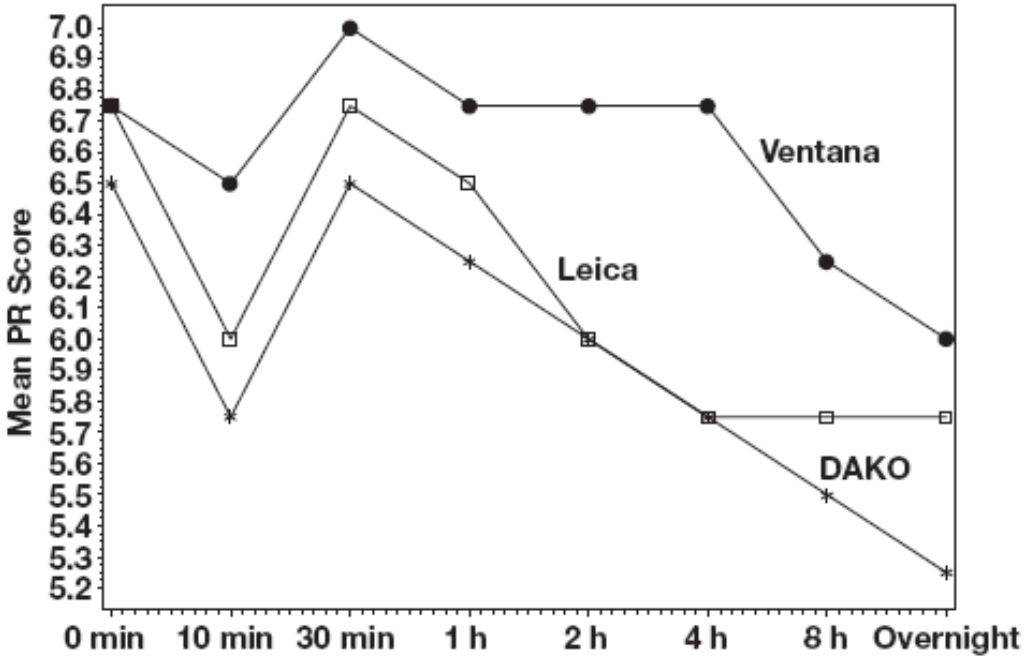
AJCP. 2010. 134:813-9

Jingxin Qiu, MD, PhD,[1] Swati Kulkarni, MD,[2] Rameela Chandrasekhar,[3] Mark Rees, PhD,[4,6] Kathryn Hyde,[5] Gregory Wilding, PhD,[3] Dongfeng Tan, MD,[6] and Thaer Khoury, MD[1]

ER: Ischemic time







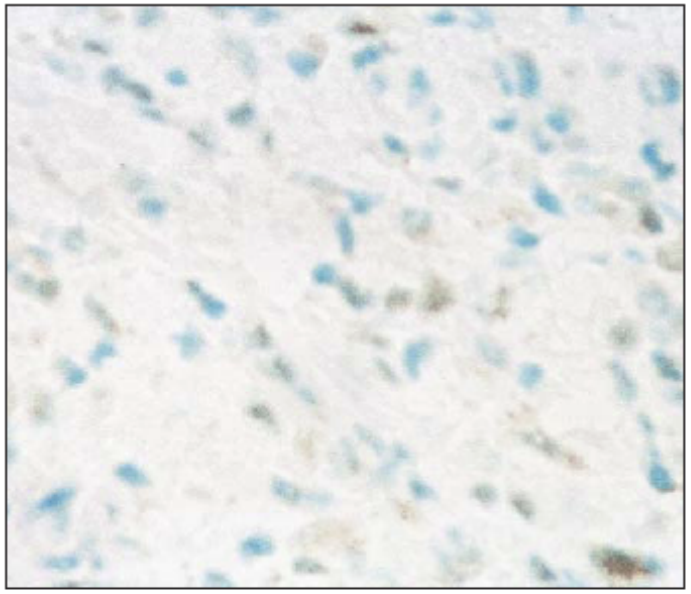


Image 1 Fixation, 3 h; antigen retrieval, 40 min.

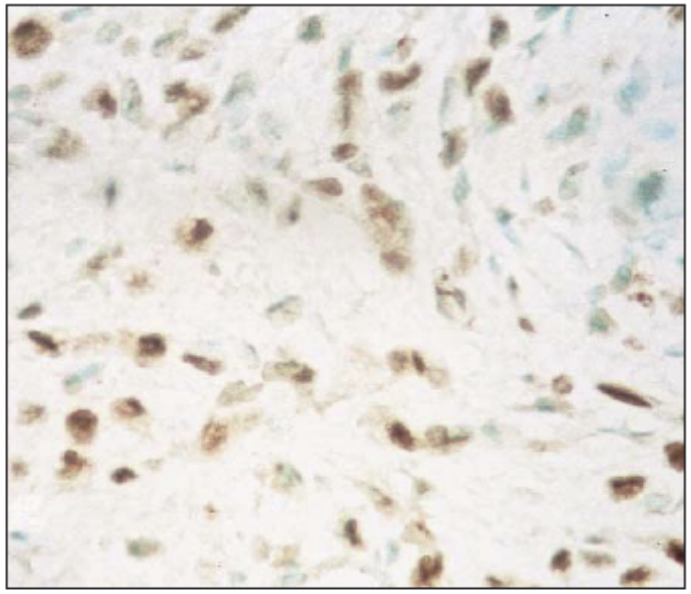


Image 2 Fixation, 6 h; antigen retrieval, 40 min.

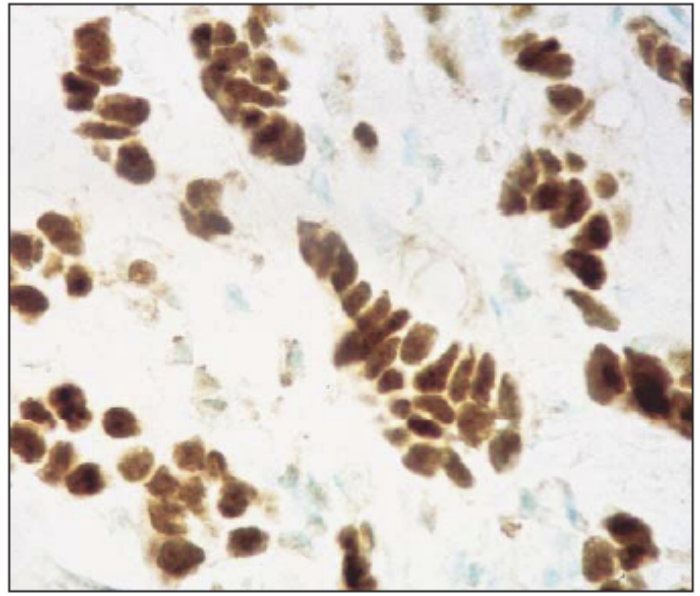


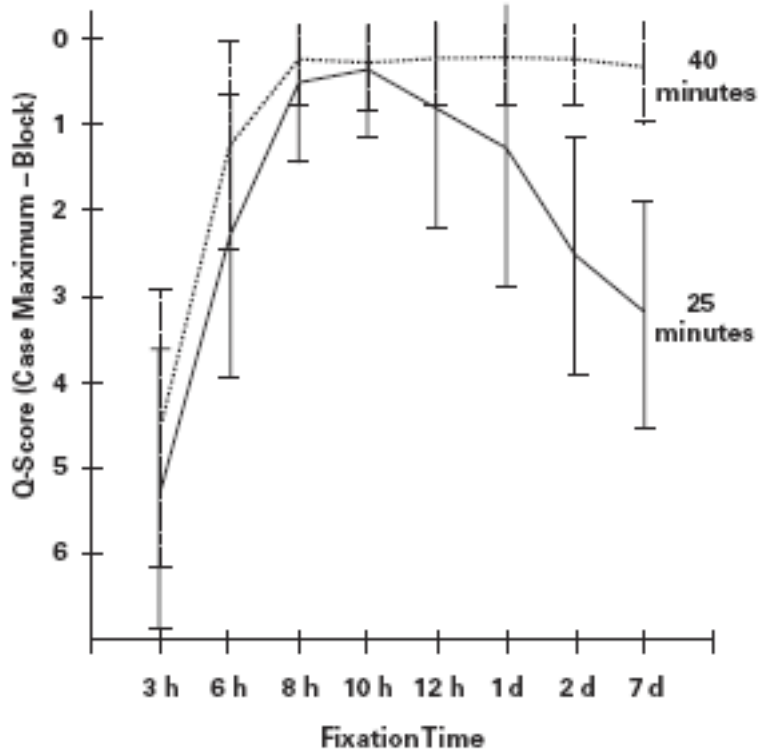
Image 3 Fixation, 8 h; antigen retrieval, 40 min.

ER: fixation time

24 Large breast tumors

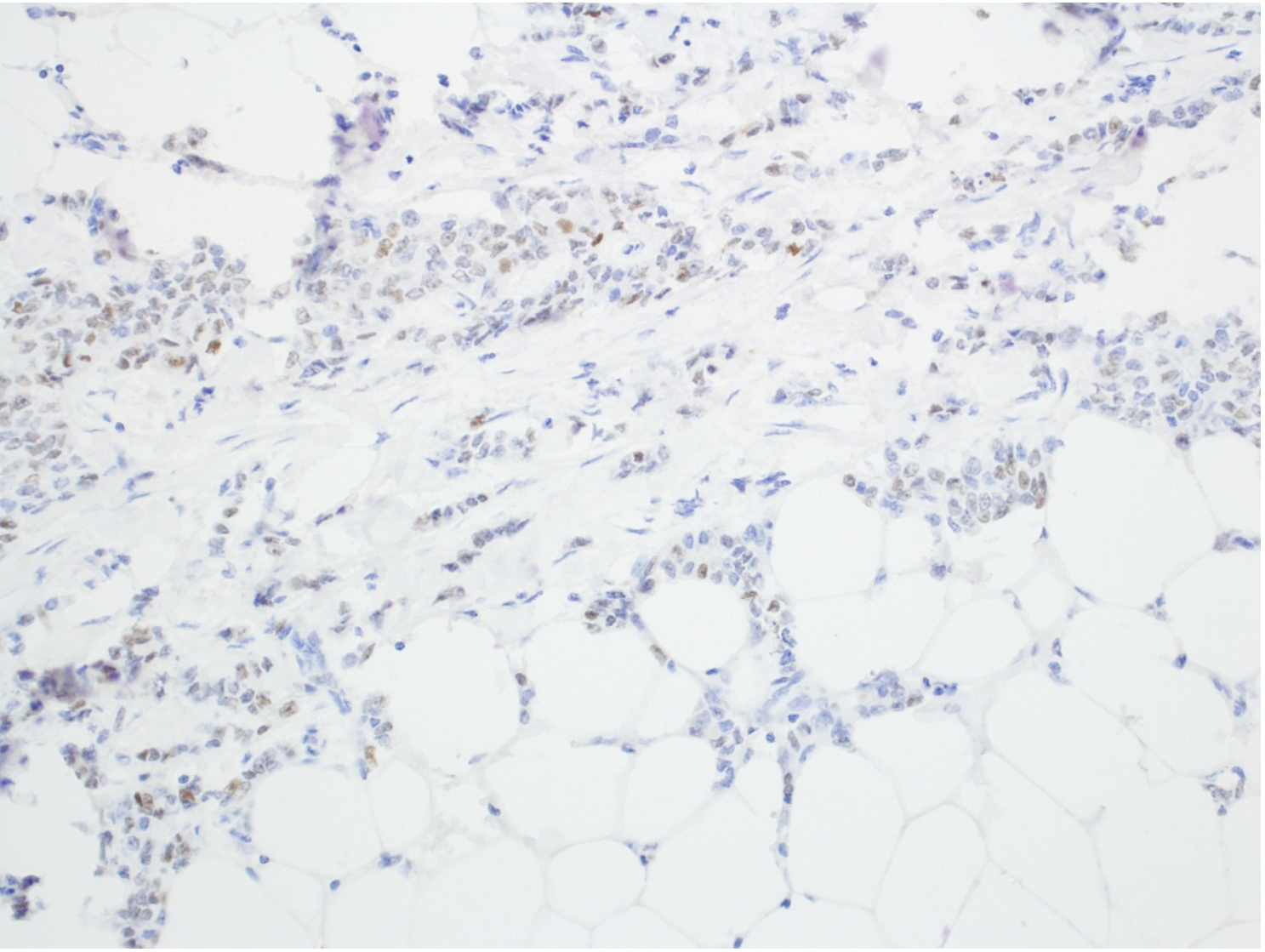
Timed fixation (3, 6, 8, 12, 24, 48, 168 hr)

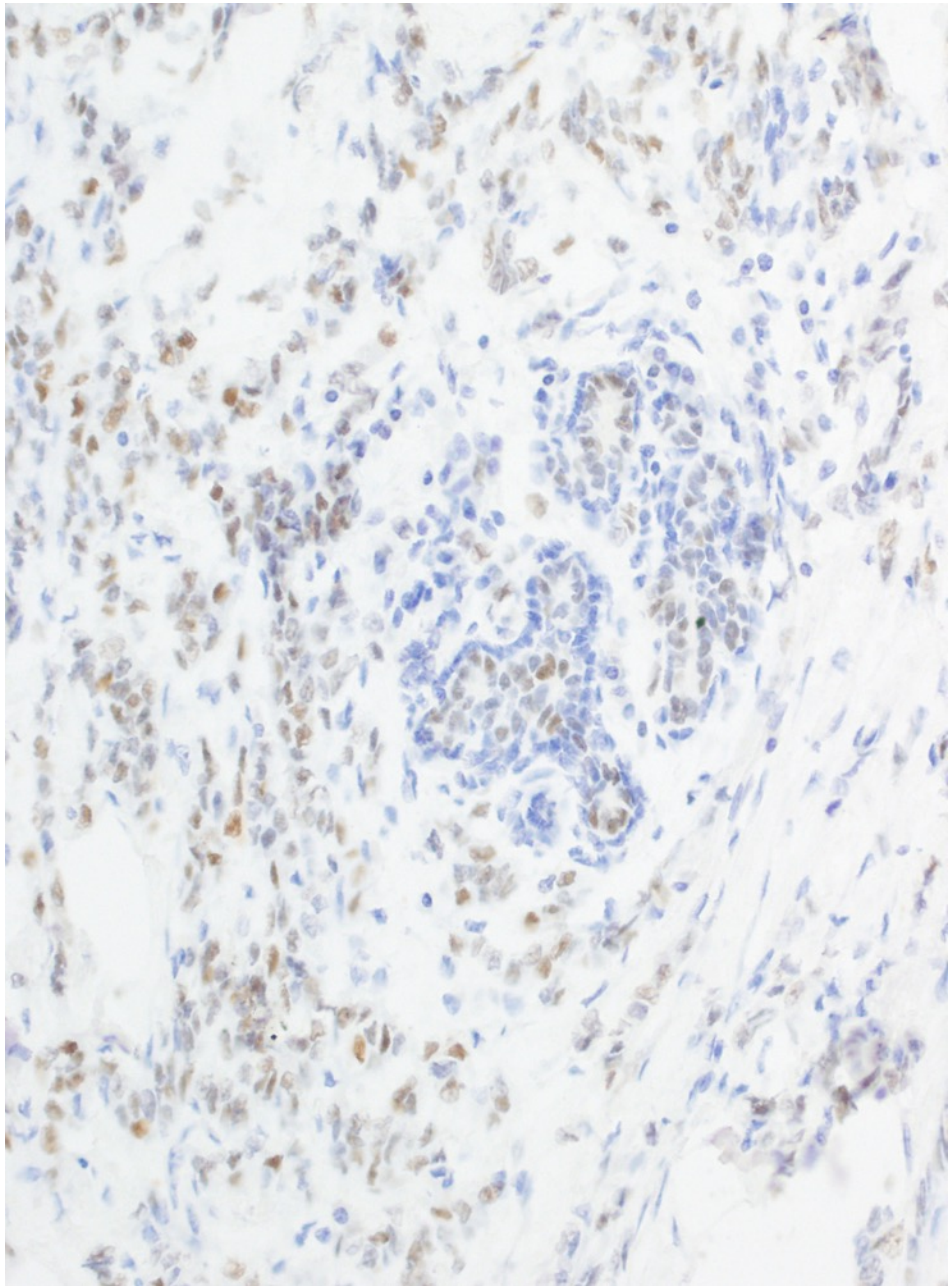
of subsamples



Goldstein et al. AJCP 120:86-92. 2003

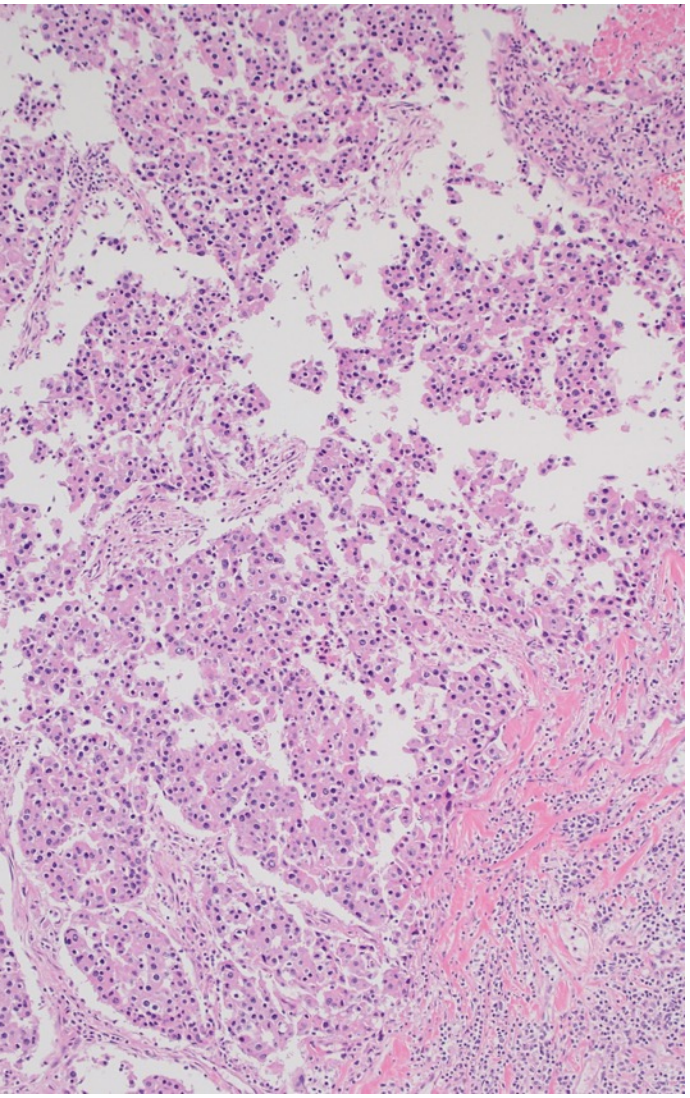
Fixation: weak ER in low grade tumor

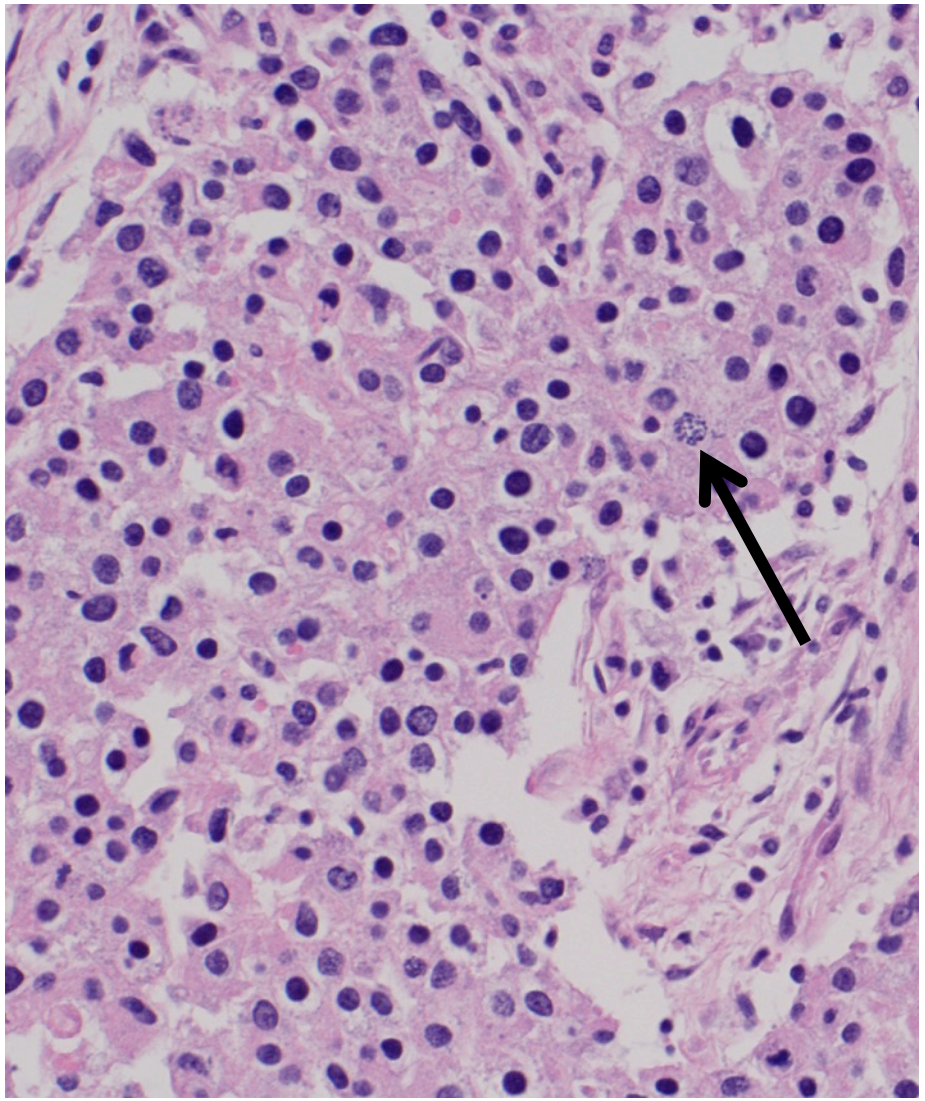


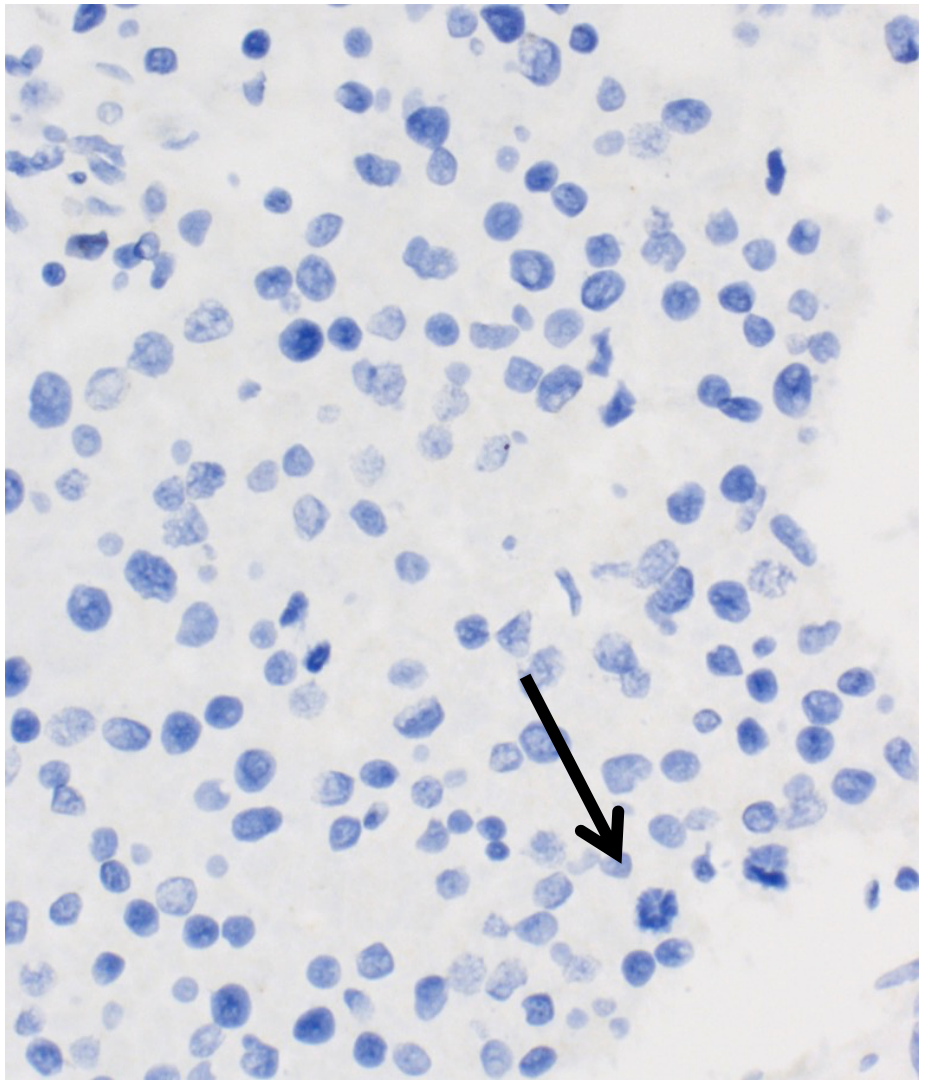


Check internal controls for ER, PR, Ki-67

Fixation: Ki-67 negative w/ mitotic figures







Check internal controls for ER, PR, Ki-67

Brief Fixation Does Not Affect Assessment of Hormone Receptor Expression in Invasive Breast Carcinoma Biopsies

Paving the Road for Same-day Tissue Diagnostics

AJSP 2014;38:1071–78

Shona Kalkman, MD, Maarten W. Barentsz, MD,† Arjen J. Witkamp, MD, PhD,‡ Elsken van der Wall, MD, PhD,§ Helena M. Verkooijen, MD, PhD,† and Paul J. van Diest, MD, PhD**

Rapid processing?

The Effects of Under 6 Hours of Formalin Fixation on Hormone Receptor and HER2 Expression in Invasive Breast Cancer

Am J Clin Pathol 2014;142:16-22

A Systematic Review

Shona Kalkman, MD,[1] Maarten W. Barentsz, MD,[2] and Paul J. van Diest, MD, PhD[1]

Brief fixation enables same-day breast cancer diagnosis with reliable assessment of hormone receptors, E-cadherin and HER2/Neu

Altuna Halilovic,[1] Joris Bulte,[2] Yvonne Jacobs,[1] Hanneke Braam,[1] Patricia van Cleef,[1] Margrethe Schlooz-Vries,[2] Annelies Werner,[2] Oliver Boelens,[3] Iris Nagtegaal,[1] Hans de Wilt,[2] Peter Bult[1]

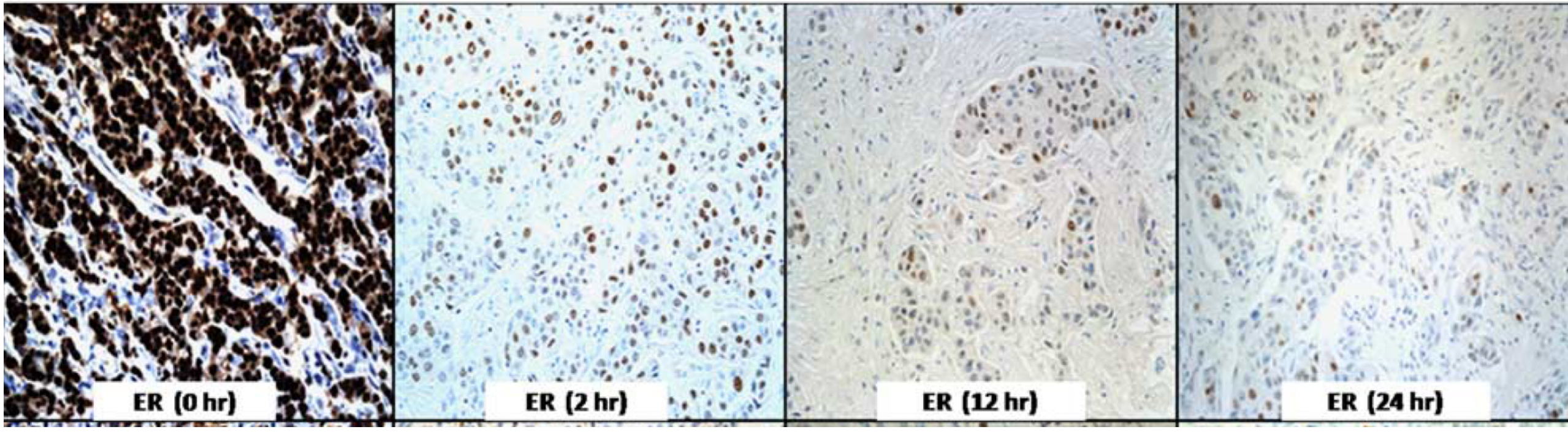
J Clin Pathol 2017;70:781–786.

Not recommended.

Effect of Hydrochloric Acid Decalcification on Expression Pattern of Prognostic Markers in Invasive Breast Carcinomas

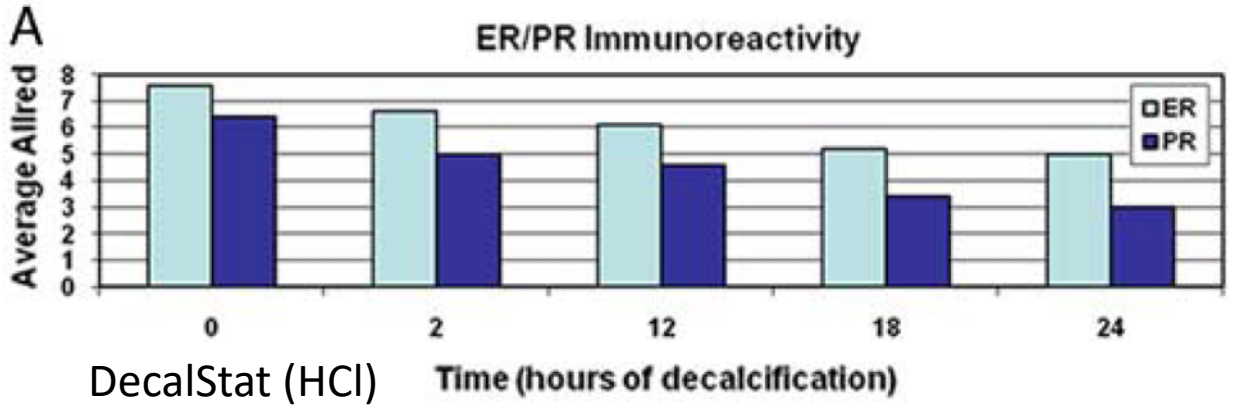
Appl Immunohistochem Mol Morphol 2017;25:144–149)

Shawn C. Maclary, PA(ASCP), MLSCM, Sambit K. Mohanty, MD, Shikha Bose, MD, Fai Chung, BS, and Bonnie L. Balzer, MD, PhD

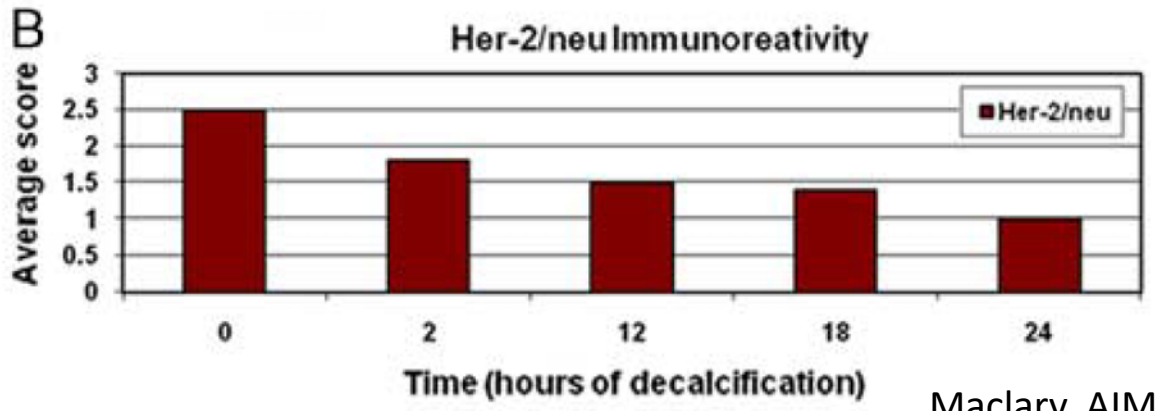


Decalcification

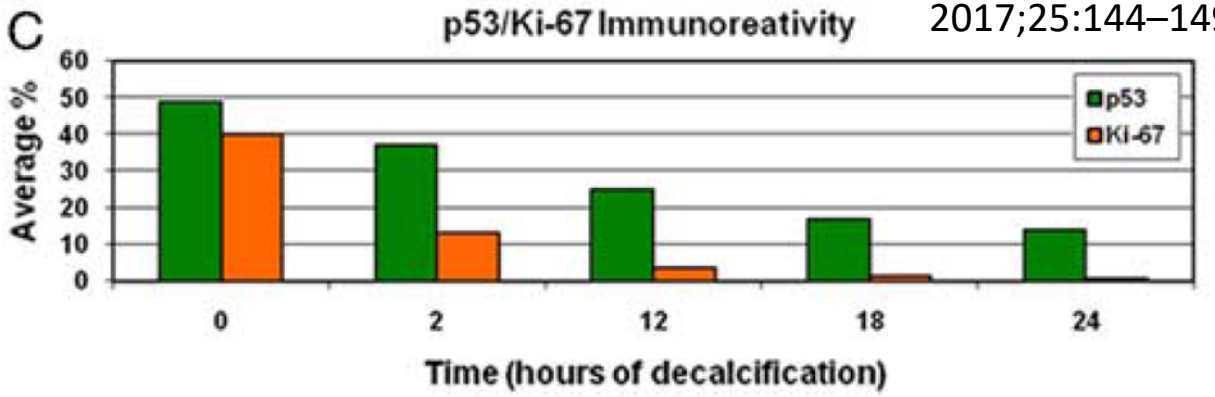
- Bone biopsies are often done to obtain biomarker data!
- Recommend separating grossly:
  - Bony fragments→ decal
  - Non-bony fragments →**NO DECAL**
  - Helpful for FISH & molecular



DecalStat (HCl)



Maclary. AIMM. 2017;25:144–149



See also: Clark. AIMM. 2019;27:223-30
Schrivjer. Mod Pathol. 2016;29:1460-70
Gertych. Diagn Pathol. 2014;9:213
Miquelestorena-Standley Mod Pathol. 2020;33:1505–17 (not breast markers)

Decalcification

- Validate **your lab's** decal/FFPE/Ab if high volume
- Evaluate internal control, if any
- Clinician request for stains on decal:
  - + result, report as not validated
  - - result, report as not validated with disclaimer re: false negative
    - CAP checklist disclaimer "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."
- Helpful to consider primary breast CA data

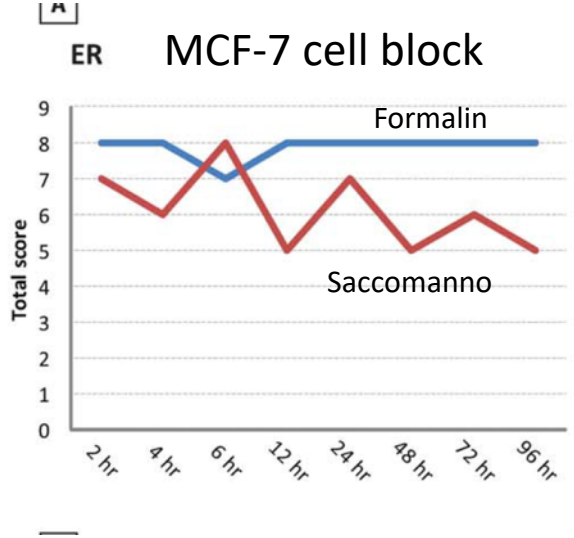
Decal	EDTA	Acetic	HCl/ Formic
ER % change	-0.5%	-2.5%	-21%
ER false neg	0	0	42%
PR % change	-1.5%	-0.5%	-14.5%
PR false neg	0	0	33%
HER2 change	-0.3	-0.3	-0.8
ISH failure	1/16	15/16	all

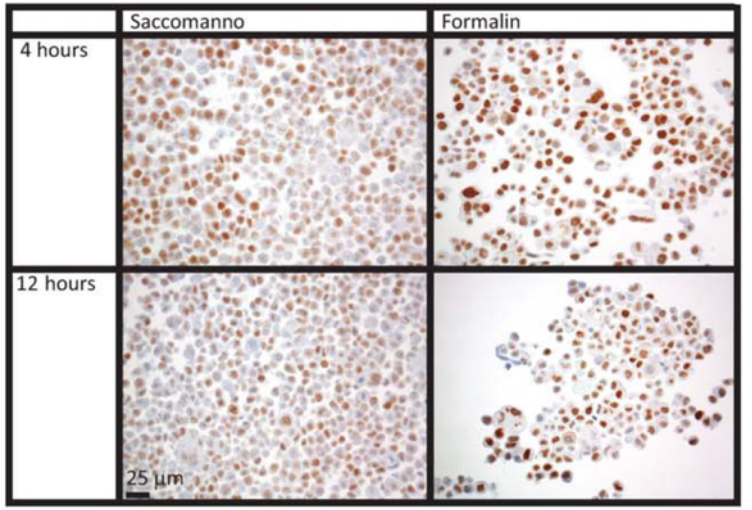
Van Es. AJSP. 2019;43:1355–60

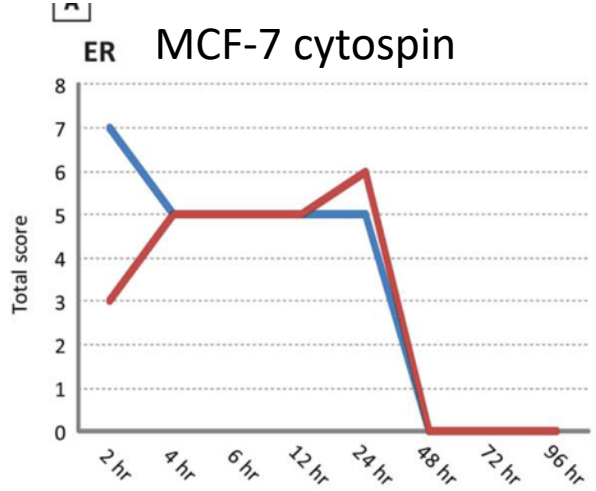
See also: Clark. AIMM. 2019;27:223-30
Schrivjer. Mod Pathol. 2016;29:1460-70
Gertych. Diagn Pathol. 2014;9:213
Miquelestorena-Standley Mod Pathol. 2020;33:1505–17 (not breast markers)

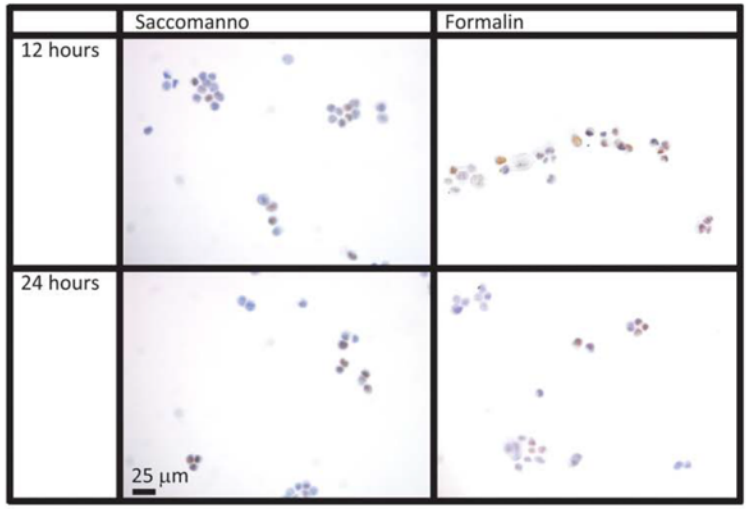
Cytologic fixative

- FNAB are often done to obtain biomarker data!
- Most cyto fixatives alcohol based
- Differ widely between labs
- Many labs use formalin-only for suspected breast metastasis, or
- Validate **your lab's** cyto fix/cell block/Ab
- Evaluate internal control, if any



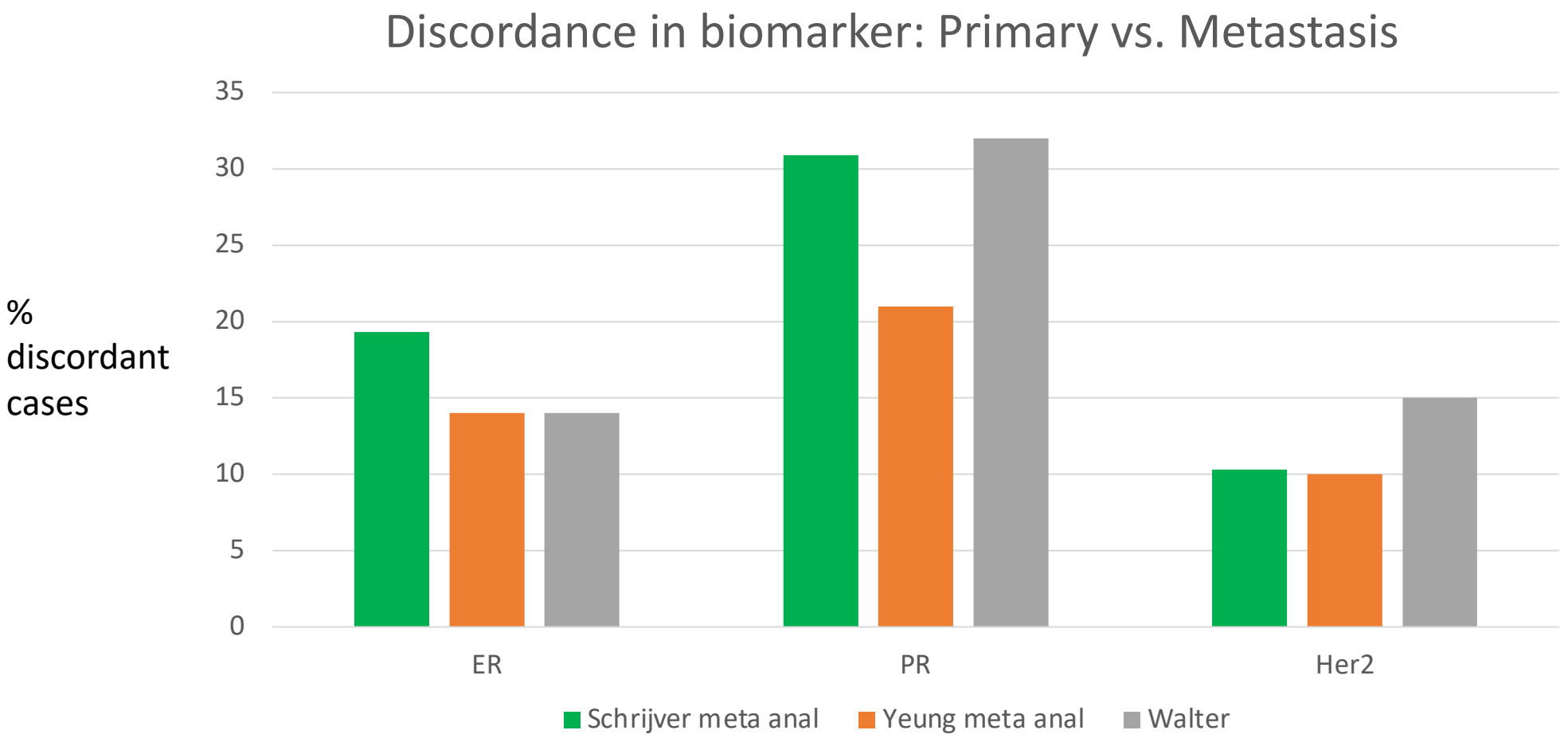






Maleki. Diagn. Cytopathol. 2013;41:864-70
(one of many various examples)

Retest metastatic disease is well-established

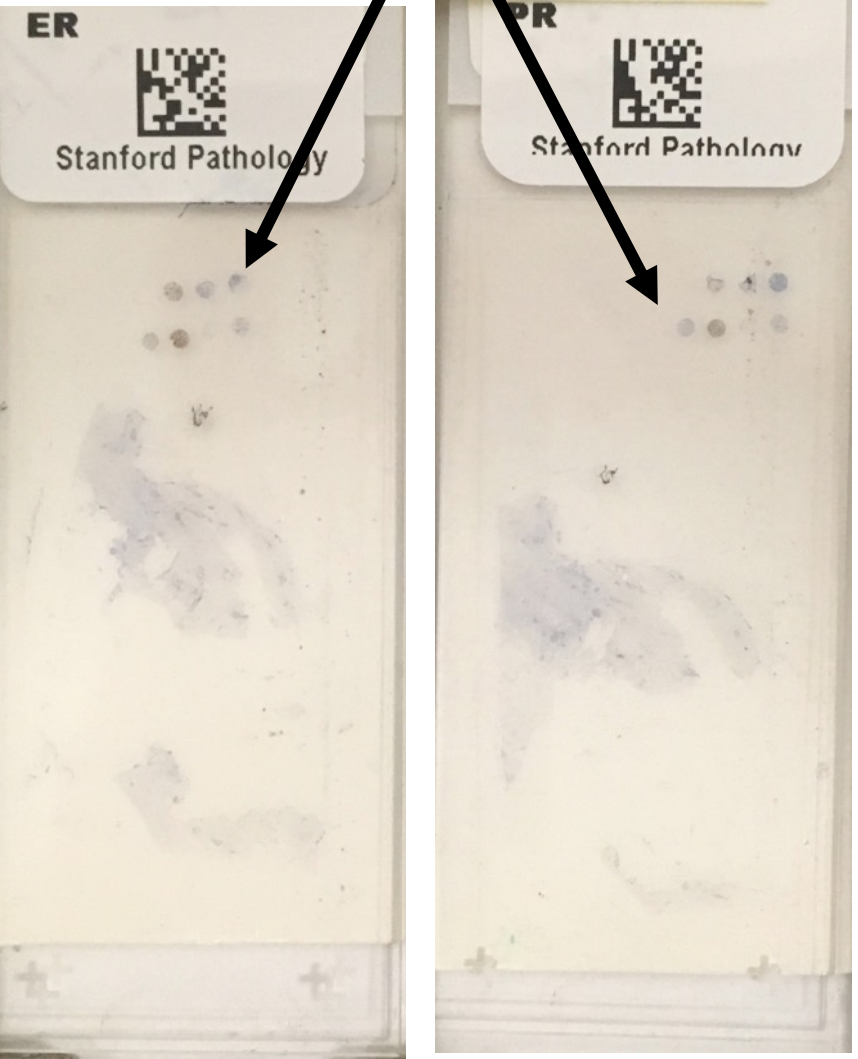


Walter. Breast Cancer Research and Treatment 2020;183:137–144

IHC, ISH and molecular are affected by pre-analytic factors in other organs also

Bass. Arch Pathol Lab Med. 2014;138:1520–30
Jones. Sci Rep. 2019;9(1):6980

External on-slide TMA or multi-tissue controls



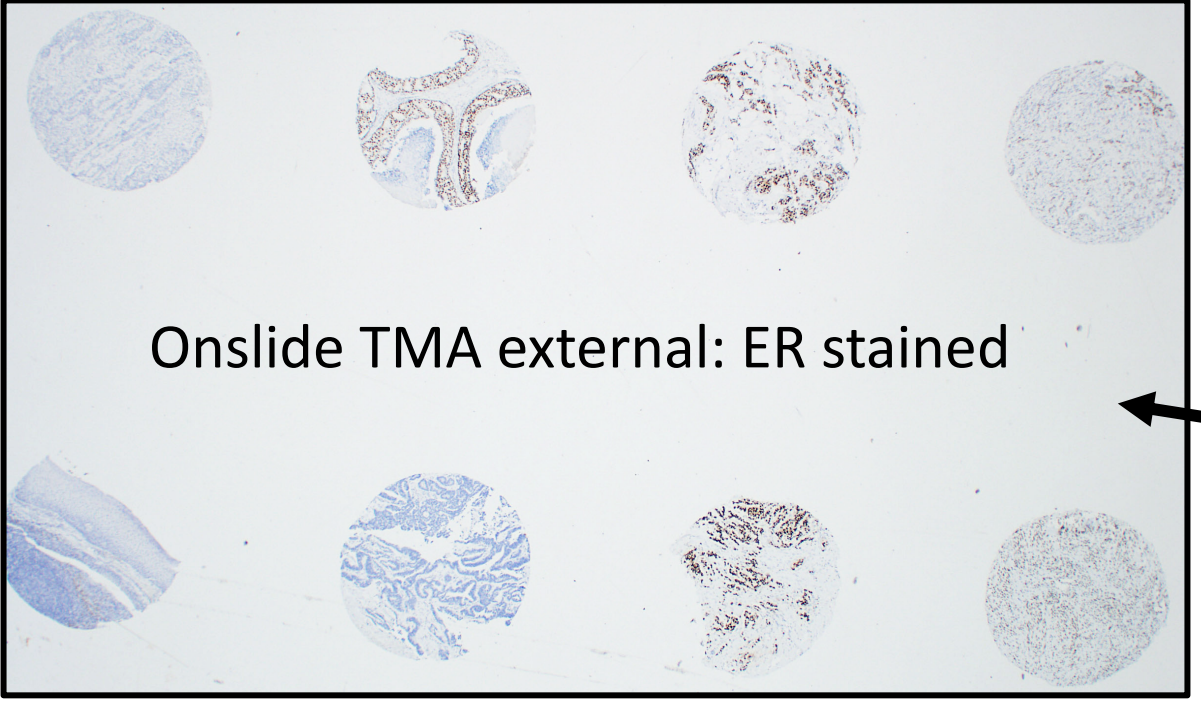
Controls & validation

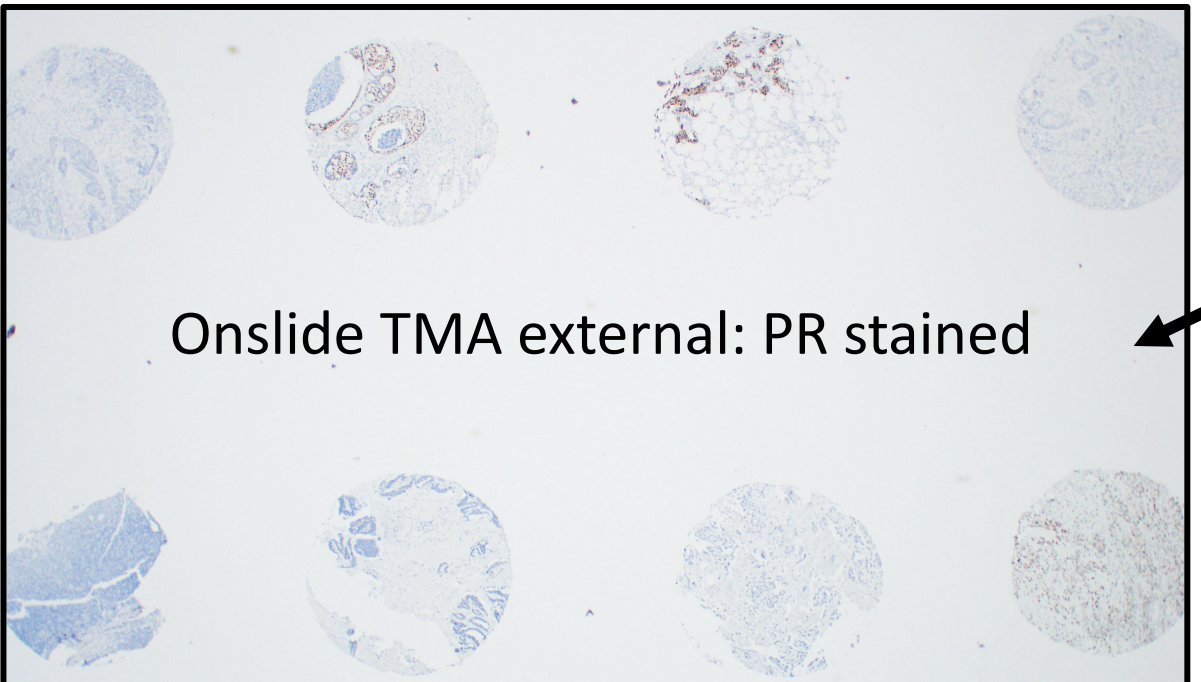
- Routine use of external controls
- Routine evaluation of internal controls
  - Including + and – samples
  - Including samples with lower % ER+ (tonsil)
  - **On slide controls** are recommended
- Assay validation: deferred to upcoming CAP IHC analytic validation guideline update
- External PT as required by accreditors (semi-annual CAP)

  →primarily based on ER

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

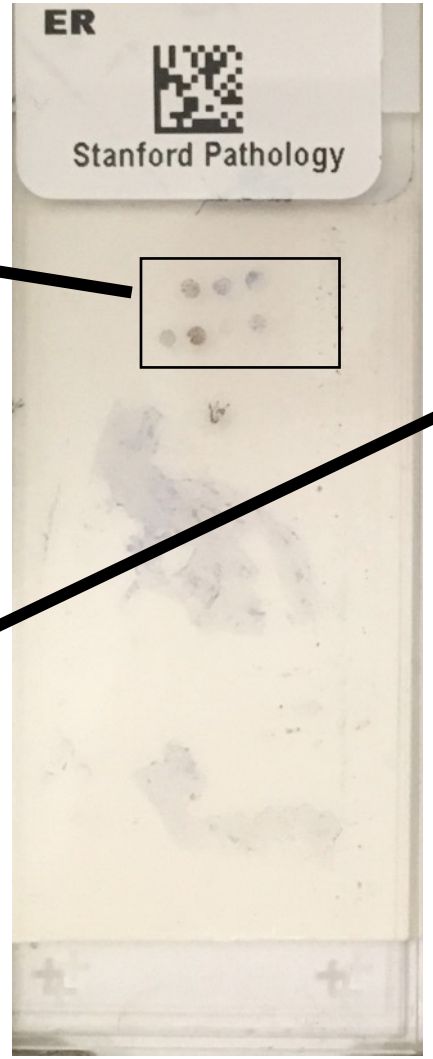
ER PR controls

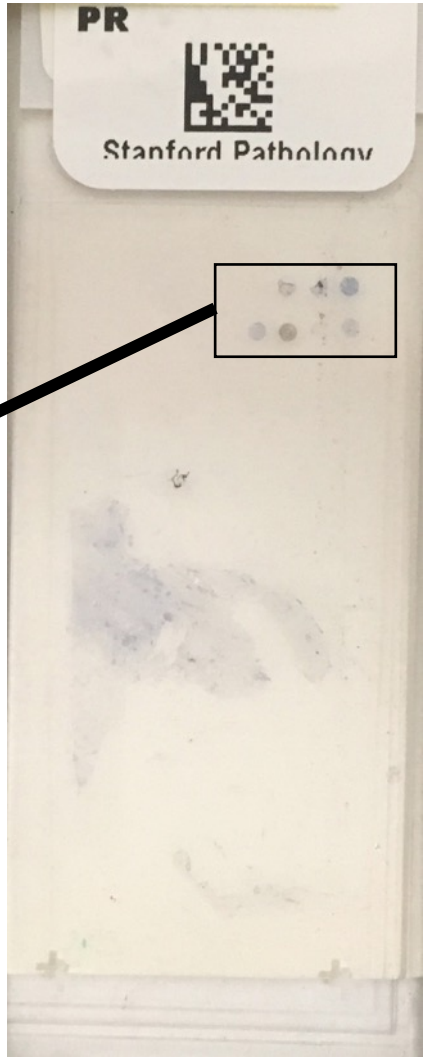




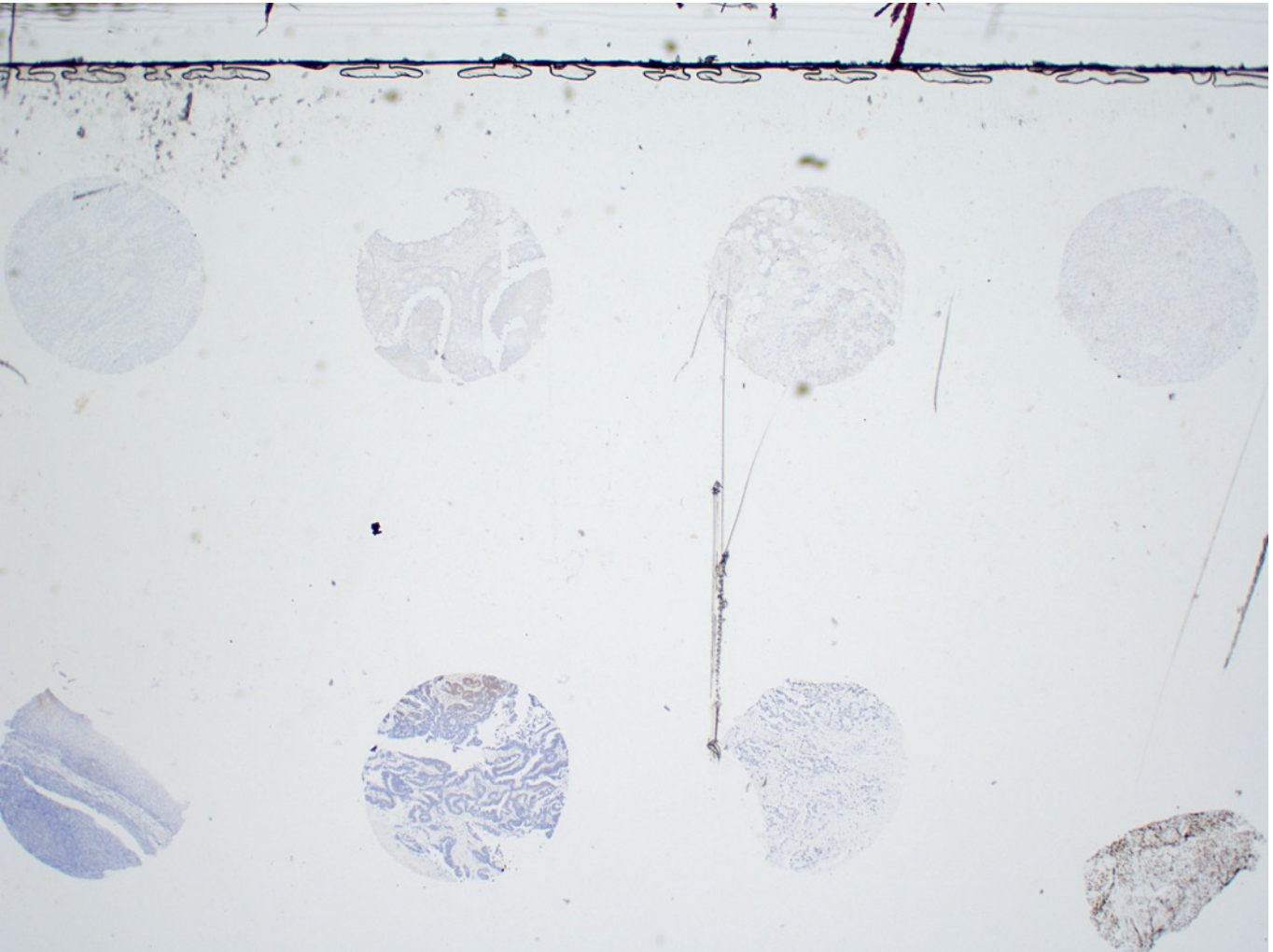
External on-slide TMA or multi-tissue controls

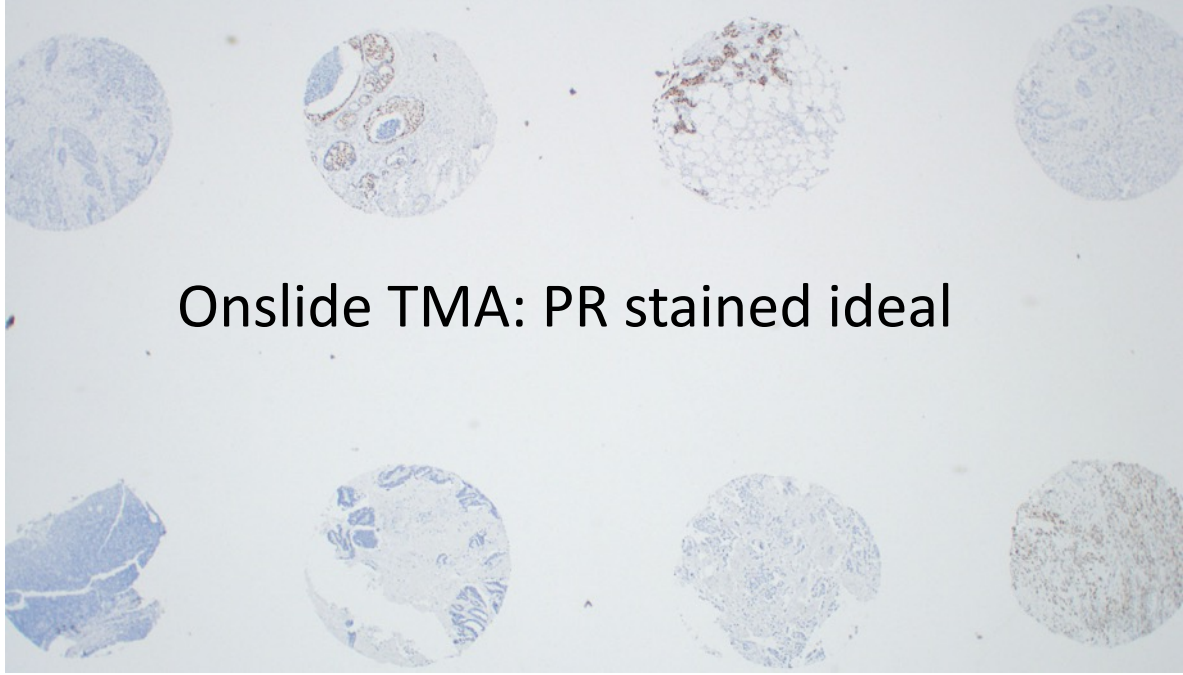
Range of + intensity



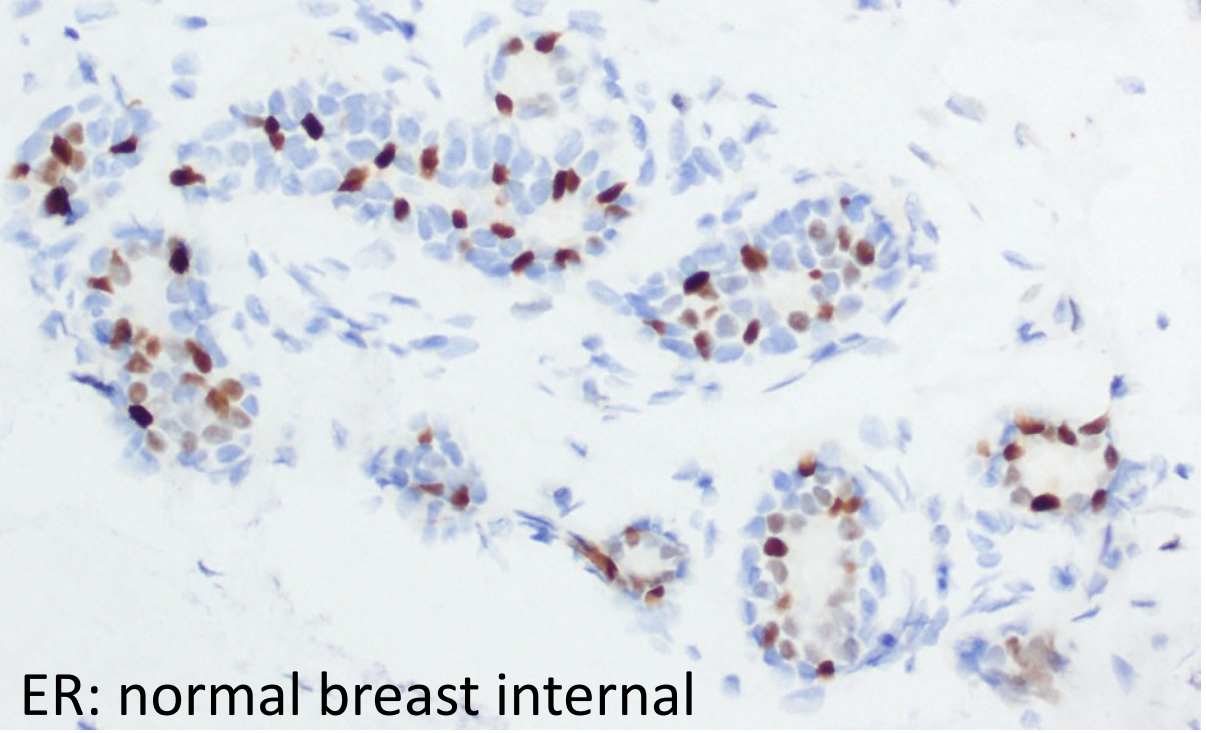


Onslide TMA: PR stained
What happened?

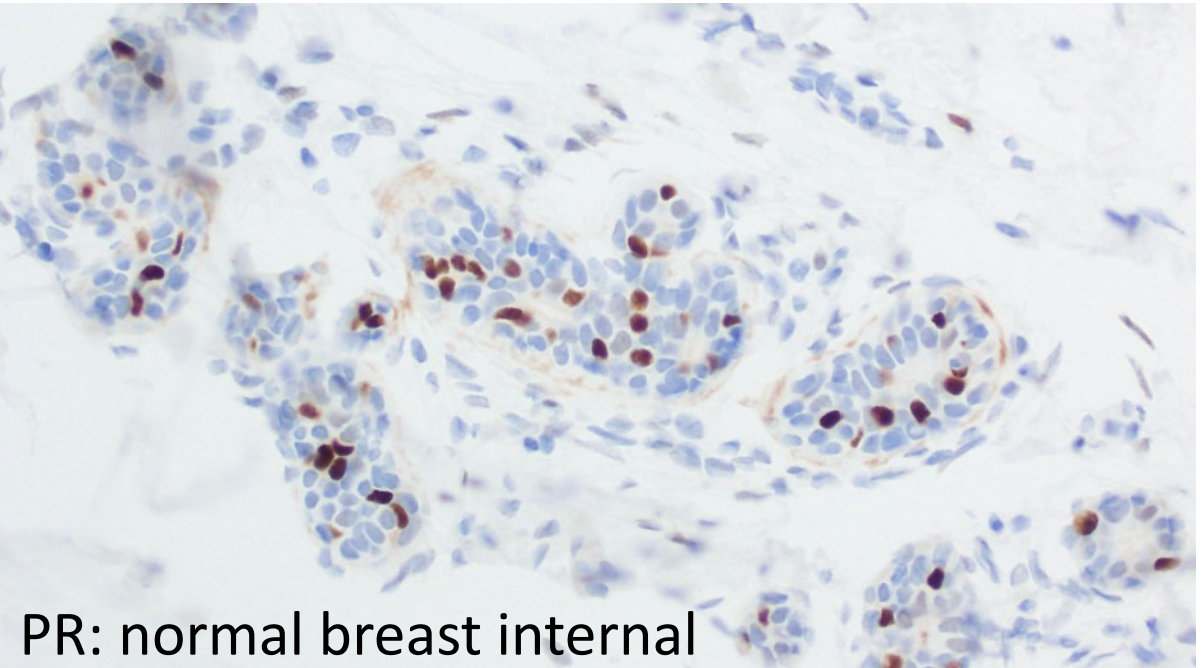




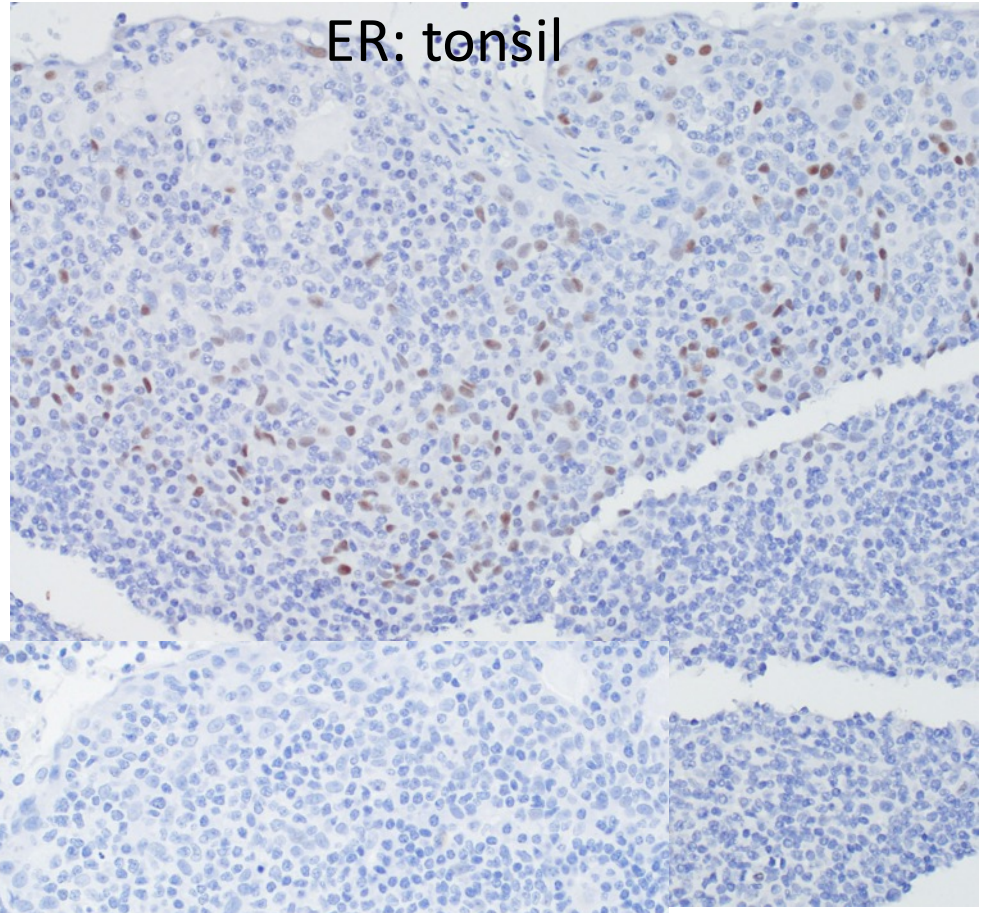
Onslide TMA: PR stained ideal



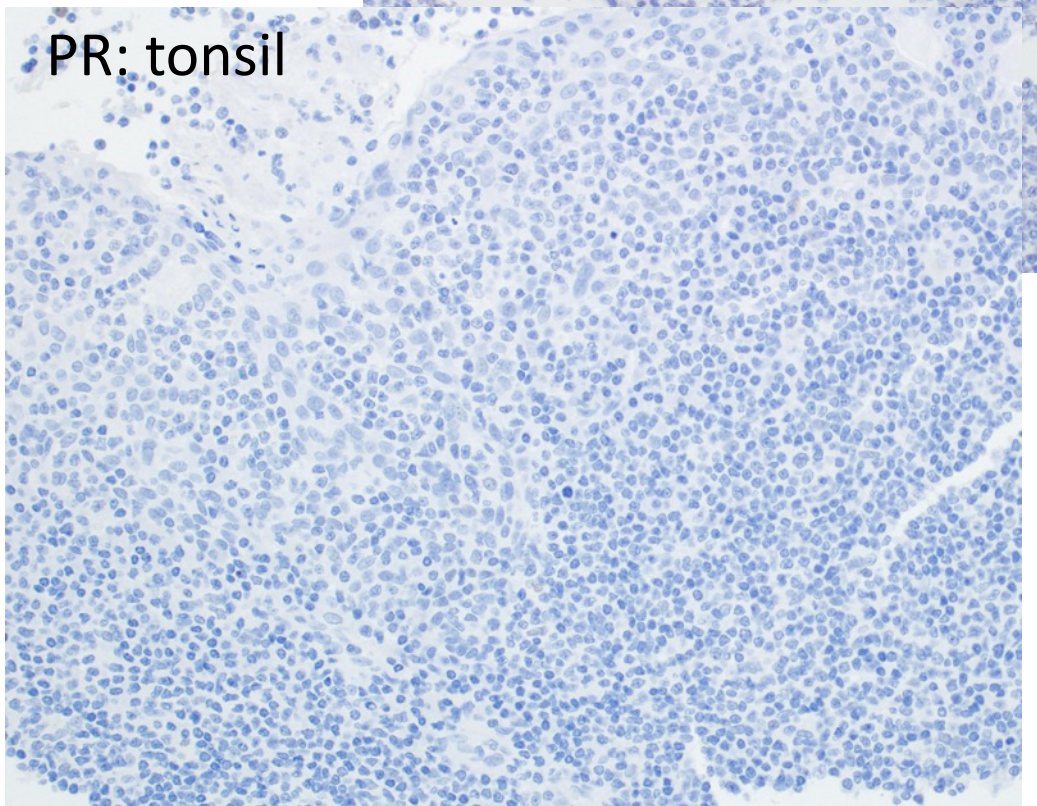
ER: normal breast internal



PR: normal breast internal



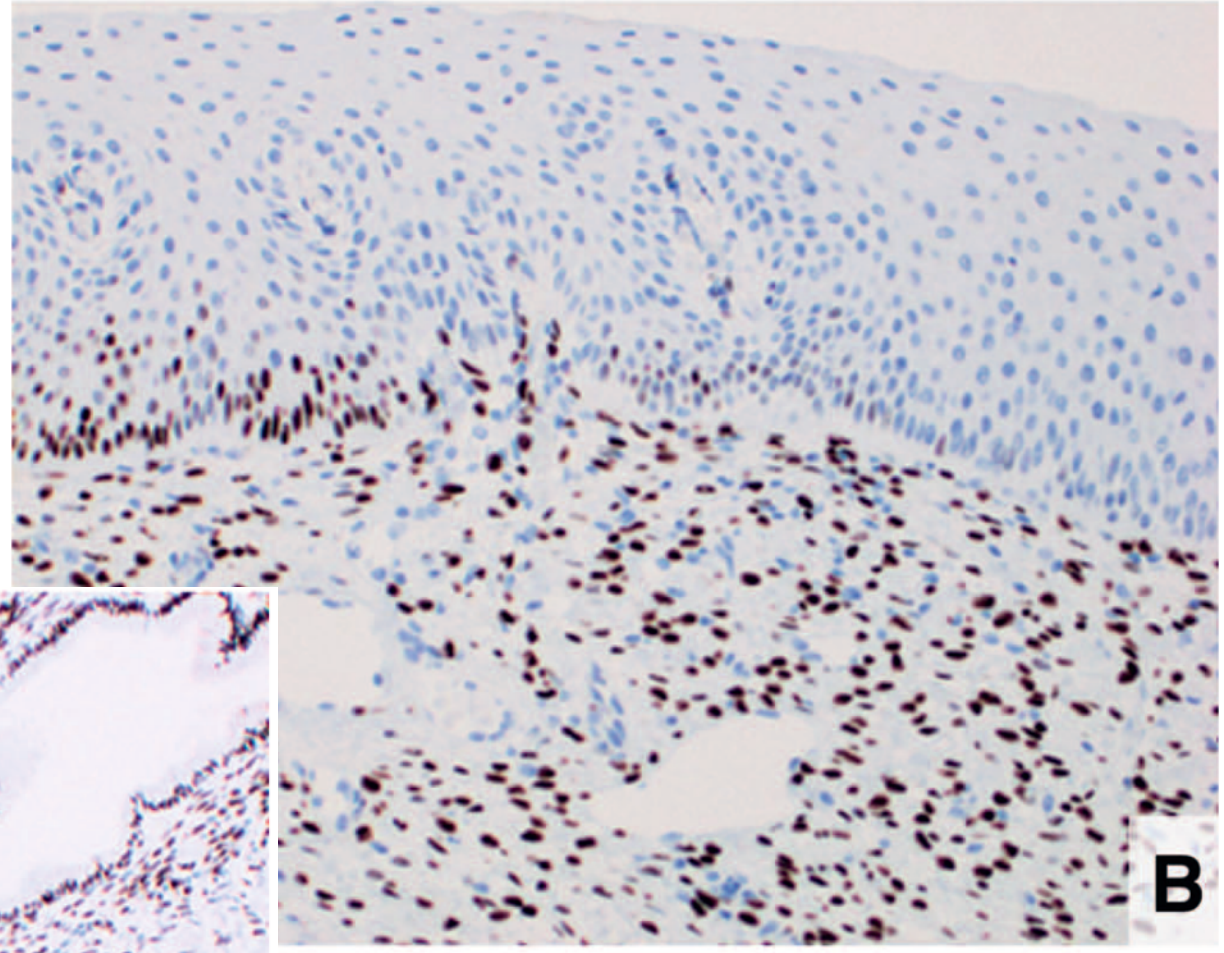
ER: tonsil

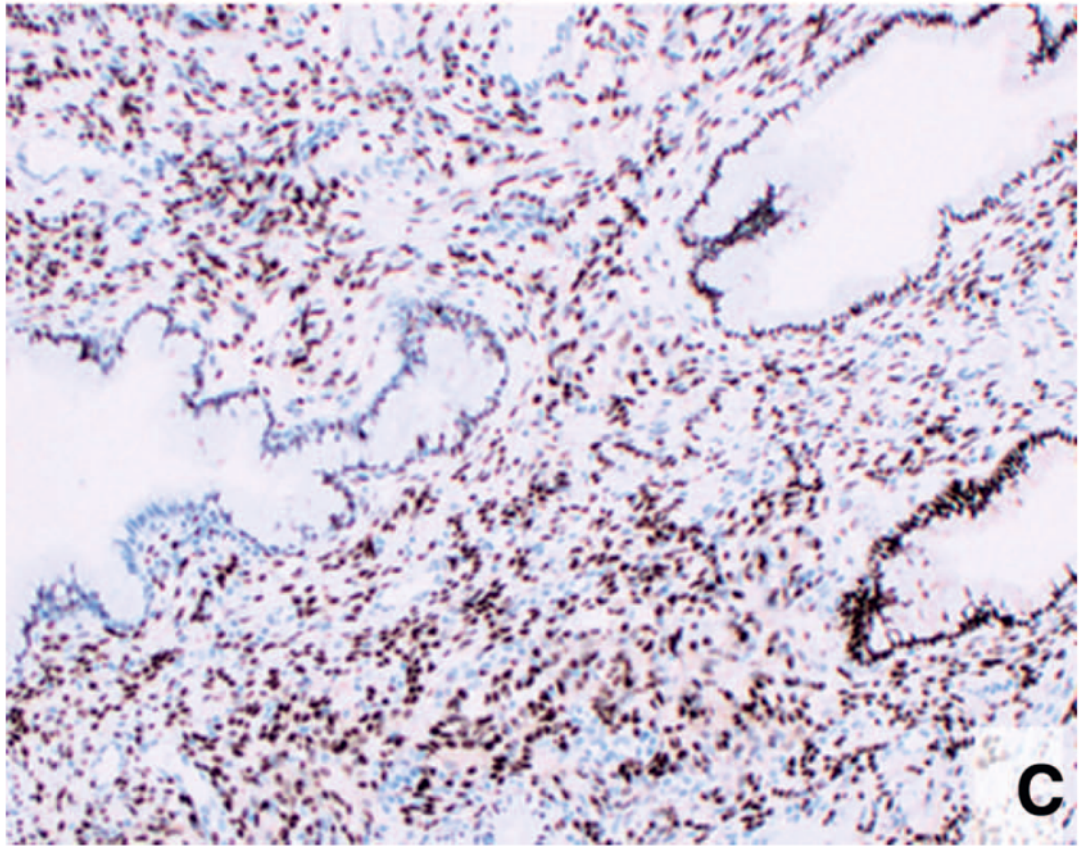


PR: tonsil

PR control: cervix

- PR stains basal squamous layer only
- PR stains stromal cells
- PR stains glands, variable

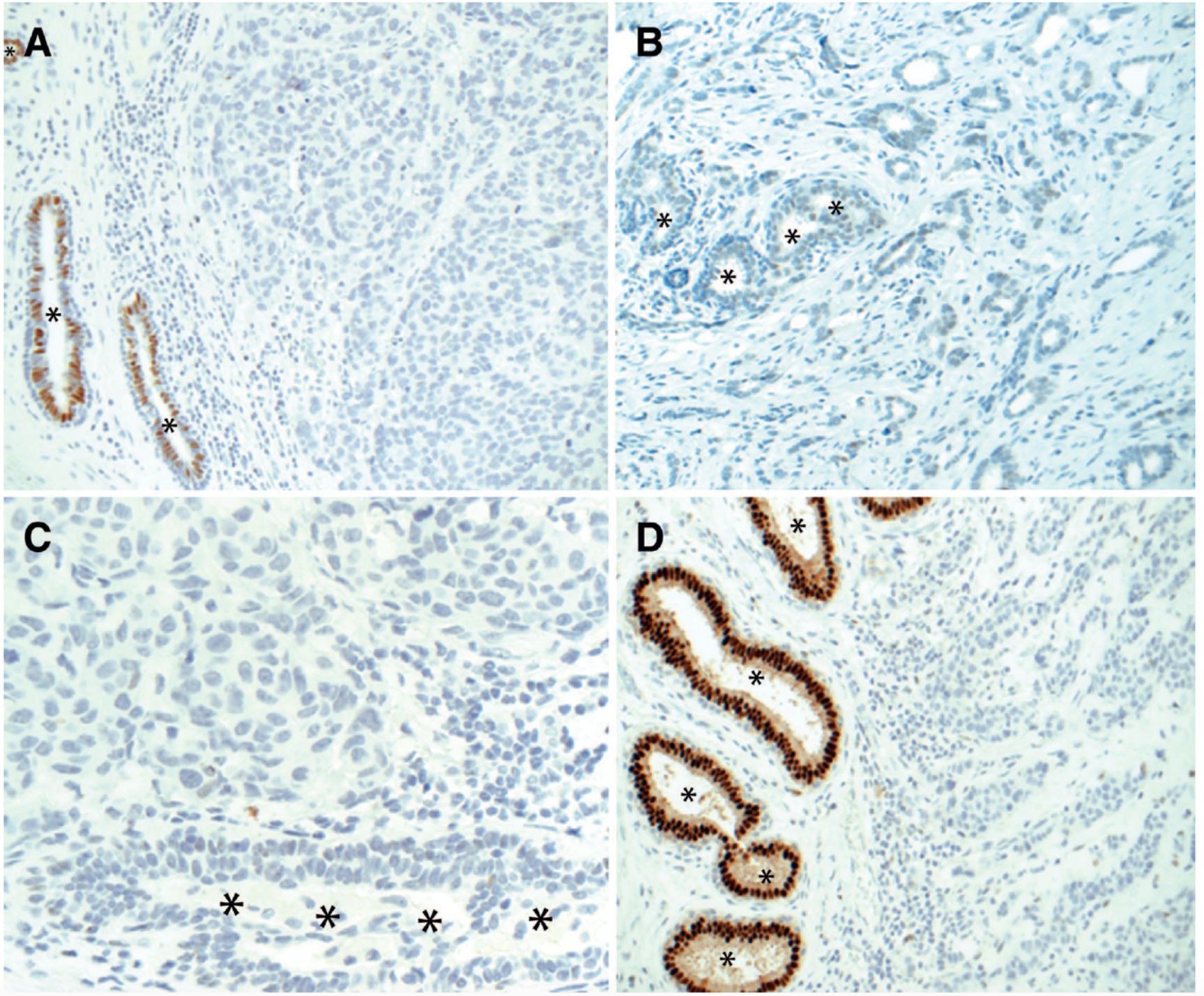




Allison. *Arch Pathol Lab Med*. 2020;144:545-563

Carcinoma & benign breast

B & C concern for weak internal control



Allison. *Arch Pathol Lab Med*. 2020;144:545-563

High Fidelity of Breast Biomarker Metrics: A 10-Year Experience in a Single, Large Academic Institution

AIMM. 2018;26:697–700

Huina Zhang, MD, PhD, Min Han, MD, PhD, Kavita R. Varma, MD, Beth Z. Clark, MD, Rohit Bhargava, MD, and David J. Dabbs, MD

Variability of predictive markers (hormone receptors, Her2, Ki67) and intrinsic subtypes of breast cancer in four consecutive years 2015–2018

Journal of Cancer Research and Clinical Oncology 2019;145:2983–94

Lidija Stevanovic[1] · Matthias Choschzick[1] · Linda Moskovszky[1] · Zsuzsanna Varga[1]

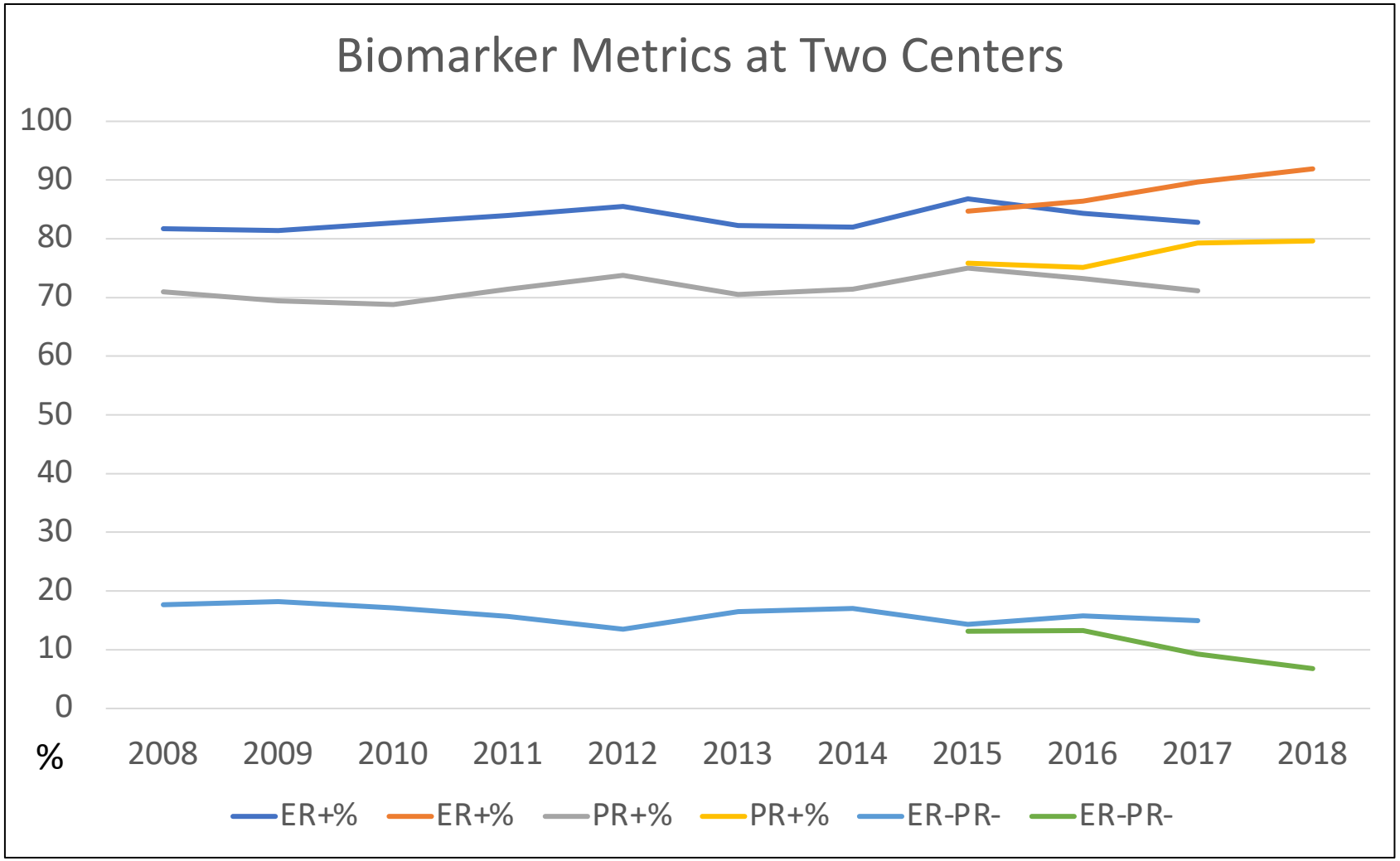
Track lab predictive marker statistics

--Internal consistency

--External benchmark %

--Differs by population

--Concordance with sendout (or RT-PCR)



COLLEGE of AMERICAN PATHOLOGISTS

Surveys and Anatomic Pathology Education Programs

ER and PgR Immunohistochemistry Tissue Microarray PM2-A 2019

Participant Summary

Progesterone Receptor Results PM2-03, cont'd

Core-03 Not Graded

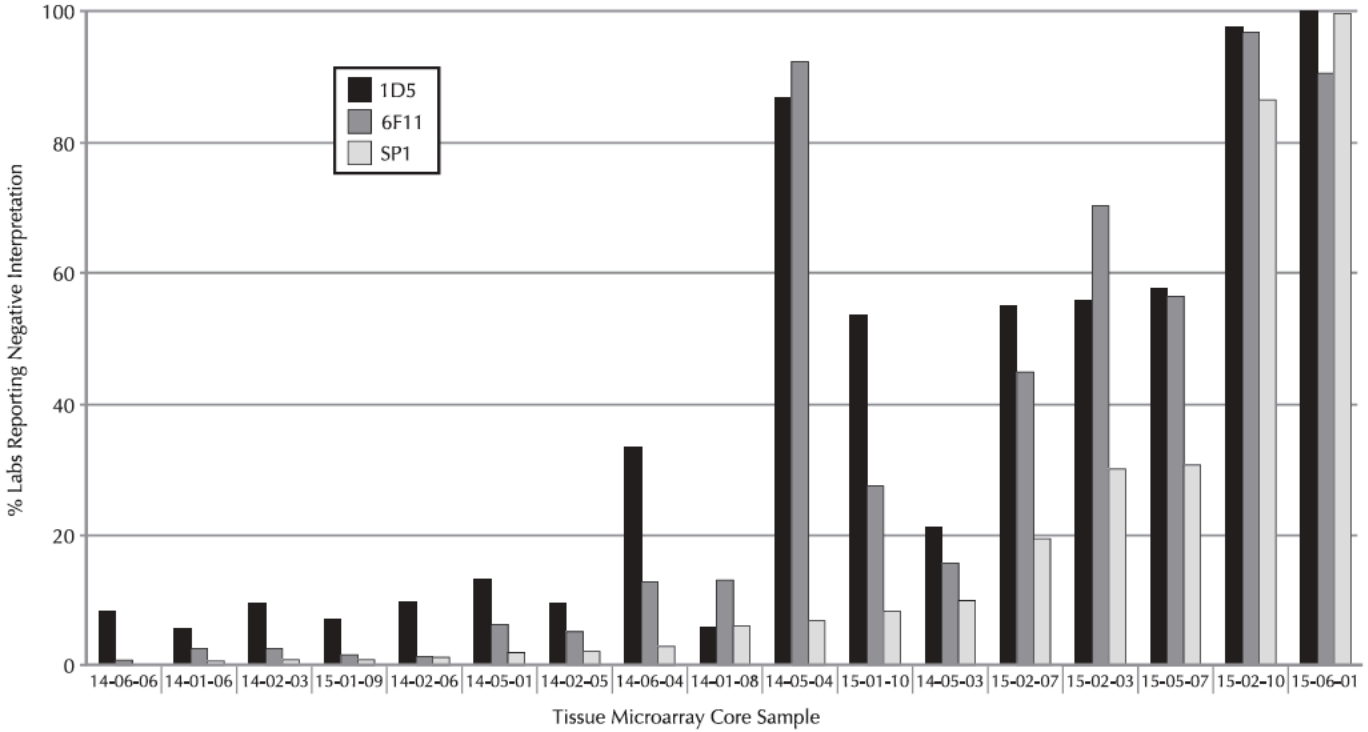
Clone	PgR Negative			PgR Positive				No Invasive Cancer Present in Core	Core Tissue not Present
	No Staining	<1%	Total (%)	1-10%	11-50%	>50%	Total (%)		
1E2 (per manuf kit inst)	73	95	168 (23.2)	358	179	20	557 (76.8)	2	-
1E2 (LDT / modified kit)	20	27	47 (29.9)	63	39	8	110 (70.1)	-	1
16	36	19	55 (24.3)	117	49	5	171 (75.7)	1	-
312	-	1	1 (7.7)	6	6	-	12 (92.3)	-	-
PgR-1A6 (1A6)	2	-	2 (50.0)	2	-	-	2 (50.0)	-	-
PgR-636 (636) (per manuf kit inst)	28	6	34 (40.5)	33	16	1	50 (59.5)	-	-
PgR-636 (636) (LDT / modified kit)	13	10	23 (35.4)	26	15	1	42 (64.6)	-	-
PgR1294 (1294)	38	19	57 (32.2)	80	37	3	120 (67.8)	-	1
Rabbit polyclonal	1	-	1 (33.3)	1	1	-	2 (66.7)	-	-
SP2	6	3	9 (37.5)	10	3	-	13 (62.5)	-	-
Y85	2	3	5 (29.6)	6	2	-	8 (70.4)	-	-
Other	4	3	7 (38.9)	8	3	-	11 (61.1)	-	-
Total (1507)	223	186	409	710	350	38	1098	3	2
Total %	14.8	12.3	27.1	47.1	23.3	2.5	72.9		

Intensity of Staining	Weak	Intermediate	Strong	Not Applicable
	771	451	39	262

PT data: a wealth of information

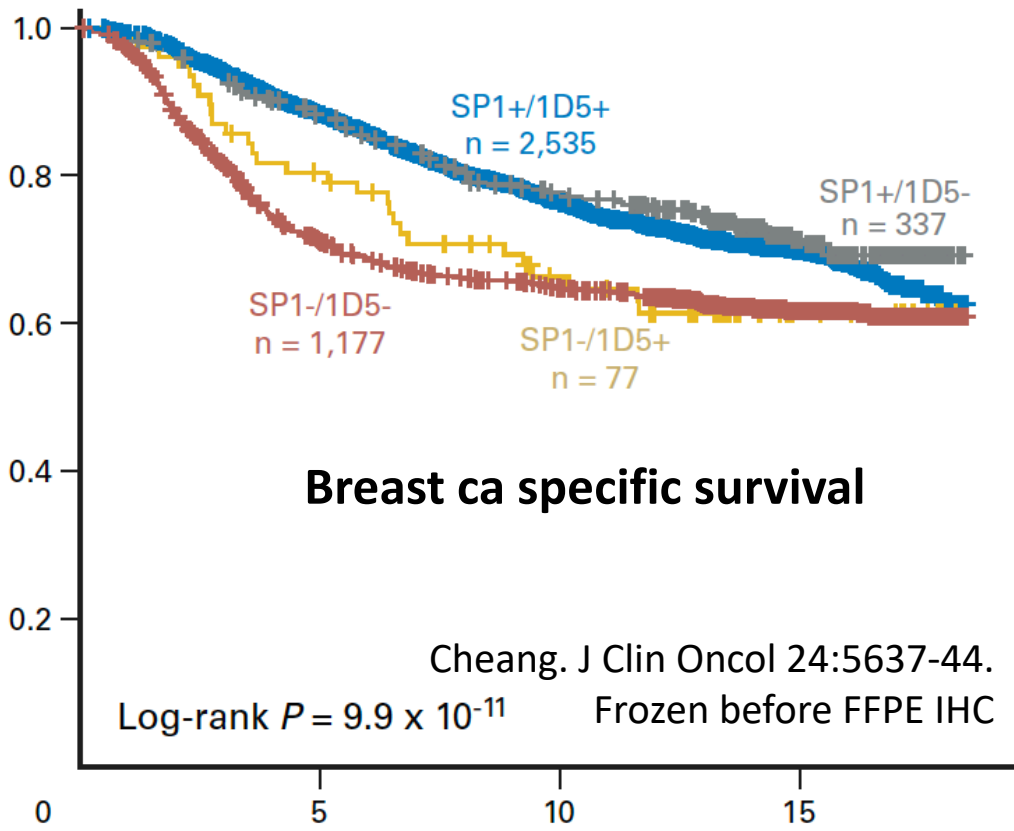
See discussion, results by core, antibody etc

ER: difference by antibody

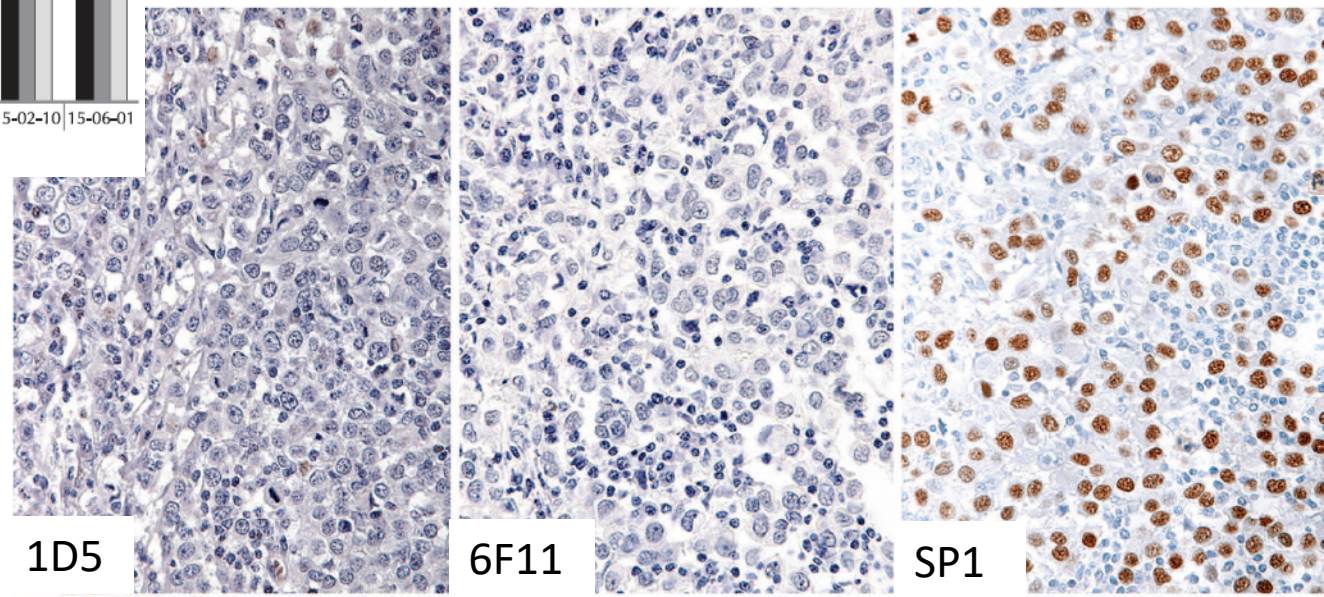


Above: ER CAP PT: TMA cores with difference by Ab (63/80 same)

Troxell. Arch Pathol Lab Med 2017;141:1402-12.



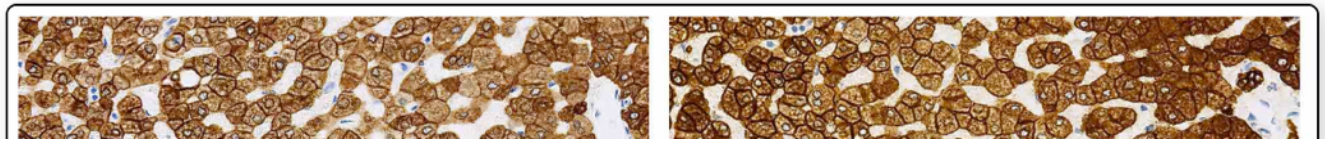
Cheang. J Clin Oncol 24:5637-44.
Frozen before FFPE IHC



NordiQC.org

Info Modules Assessments Protocols Controls Events Login

EQA handy ref



Events

4th NordiQC Conference on Applied Immunohistochemistry

ER	Estrogen Receptor	Run B28	2019	Link
ERG	Ets-Related Gene	Run 50	2017	Link
FVIII	Factor VIII related antigen	Run 11	2004	
GATA3	GATA3	Run 54	2018	Link
GCDFP	Gross cystic disease fluid protein-15	Run 36	2012	Link
GFAP	Glial fibrillary acidic protein	Run 13	2005	
GLP3	Glypican 3	Run 42	2014	Link
HCG	Human chorionic gonadotropin	Run 11	2004	
HEPA	Hepatocyte antigen	Run 36	2012	Link
HER2 IHC	HER2 IHC	Run B28	2019	Link
HER2 ISH	HER2 ISH	Run H16	2019	

central DNA-binding domain, the hormone-binding domain at the C-terminal, and the transcription-activating domain at the N-terminal. ER mediates regulatory functions of female sex steroids, mainly 17 (E2), on growth, differentiation and function in several target tissues, including female and male reproductive tract, mammary gland, and skeletal and cardiovascular systems. Recently, a second estrogen receptor, termed ER?, was discovered. Human ER? shares a high structural homology with the previously known human ER, now termed ER?, especially in the DNA- and hormone binding domains. Both receptors bind hormones with similar affinity and their transcriptional activation is identical. The tissue distribution of ER? is similar to that of ER? with some differences. In normal and malignant human breast tissue ER? is expressed in stromal cells in addition to epithelia. Only limited data are available on the role of ER? in normal and neoplastic tissues.

Neoplasms

ER? is mainly expressed in tumours of female sex steroid hormone responsive tissues such as the mammary gland, endometrium, and ovary. ER? protein is expressed in 60-70% of female breast cancers (ER+/PR- 19-22%; ER+/PR+ 49-53%). Other tumours expressing ER? are meningiomas, salivary gland tumours, some neuroendocrine tumours, and some colorectal and hepatocellular carcinomas.

Application

The applications of immunohistochemical demonstration of ER? ar
clinical use of ER? immunohistochemistry is prediction of response
carcinoma. Tumours expressing both ER? and PR react positively to
in 50-70% of cases as against below 10% of those negative for E
these facts and a number of meta-analyses, adjuvant antie
administered in most countries to postmenopausal women with ER+
(and PR) status can be used to estimate disease-free and over
immunohistochemical assay, positive steroid hormone status has
treatment. Secondly, ER? can be used as a tumour marker (see N
Progesterone receptor, e.g., in the classification of adenocarcinomas.

Controls

Uterine cervix and tonsil can be recommended as positive tissue controls for ER. In uterine cervix, virtually all squamous and columnar epithelial cells must show a moderate to strong and distinct nuclear staining reaction. Lymphocytes and endothelial cells must be negative.
Tonsil is especially found recommendable as a tool to monitor the level of analytical sensitivity for the demonstration of ER. Dispersed follicular dendritic cells in germinal centers and squamous epithelial cells must show an at least weak but distinct nuclear staining reaction. In addition, tonsil can be used as negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

Assessments

ER runs

Run B28
Run B27
Run B26
Run B25
Run B24
Run B23
Run B21
Run B19
Run B17
Run B13
Run B10
Run 13
Run B07
Run 10
Run B03
Run B08
Run B15

Each run links to pdf with detailed Analysis of protocols and examples of good and poor result

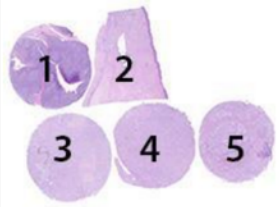
Assessment Run B28 2019

Estrogen receptor (ER)

Material

The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Tonsil	1–5%	Weak to moderate
2.	Uterine cervix	80-90%	Moderate to strong
3.	Breast carcinoma	40-60%	Weak to moderate
4.	Breast carcinoma	90-100%	Moderate to strong
5.	Breast carcinoma	Negative	-



*ER-status and staining pattern as characterized by the NordiQC reference laboratory using the rmAb clones EP1 and SP1.

What about PR?

Predictive

- Higher response to endocrine Rx if ER+/PR+ in metastatic, neoadjuvant settings
- No difference in benefit by PR status in adjuvant setting

Prognostic

- Lower PR, poorer prognosis
- PR helps forecast intrinsic type
- PR is element of IHC4, Magee equations, nomograms

- "**Continue to recommend routine PR testing of invasive**…"
  - Use 1% as positivity threshold
  - Report % and intensity
  - No Low PR category
  - PR optional for DCIS

Allison. *Arch Pathol Lab Med*. 2020;144:545-563

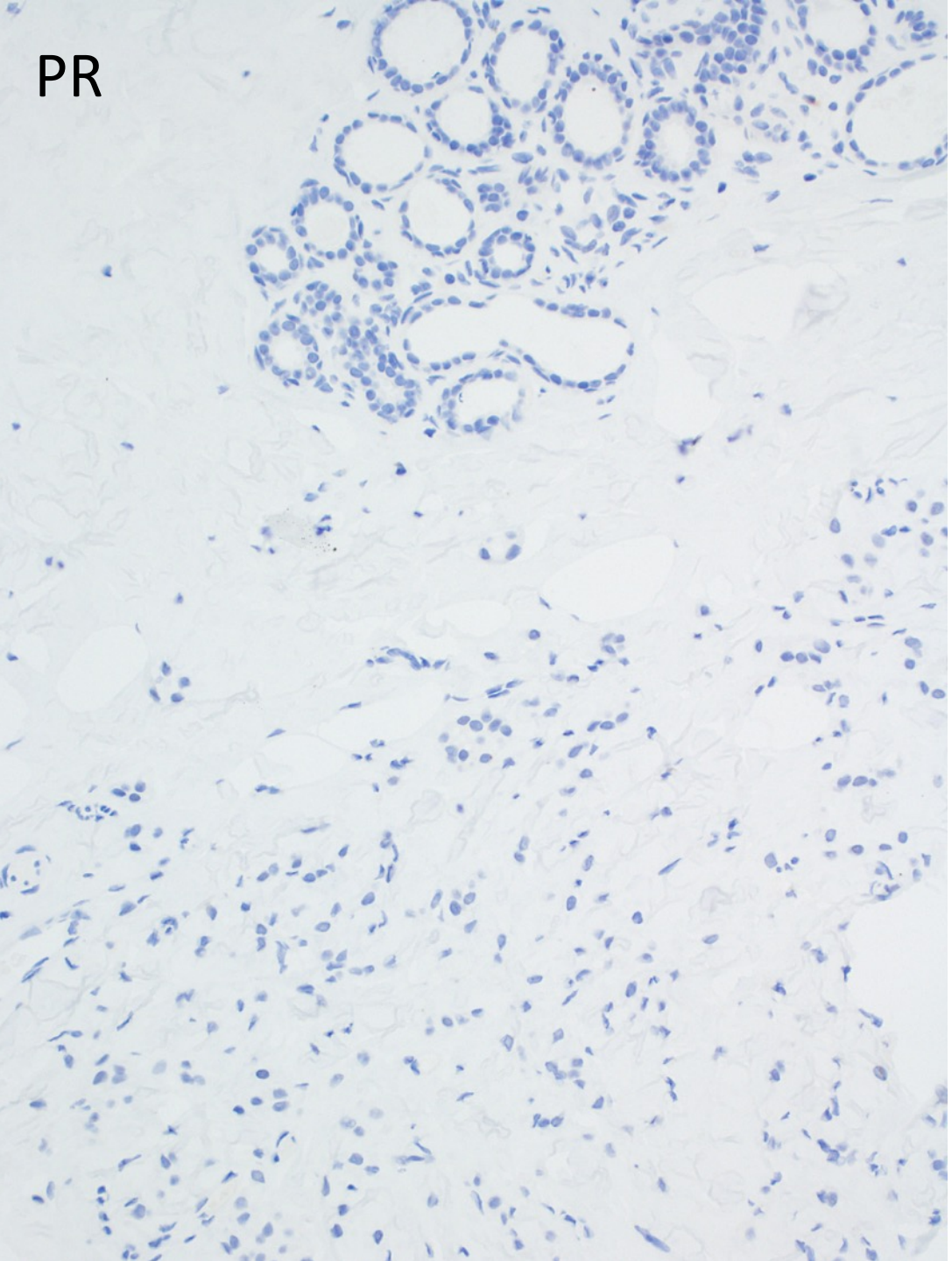
Does ER-/PR+ breast cancer exist?

- False negative ER in a truly ER+ tumor?
  - Check controls; consider repeat
- False-positive PR in an ER-PR- tumor?
- Tumor heterogeneity?
- ER-/PR+ as a rare subgroup?

- Add endocrine Rx to chemo as per TN breast cancer?

	ER+/PR+ (45%)	ER+/PR- (15%)	ER-/PR+ (4%)	ER-/PR- (37%)
LumA	59%	29%	**15%**	1%
LumB	23%	30%	**5%**	2%
Basal	6%	18%	**65%**	80%

Itoh. Breast Cancer Res Treat (2014) 143:403–409

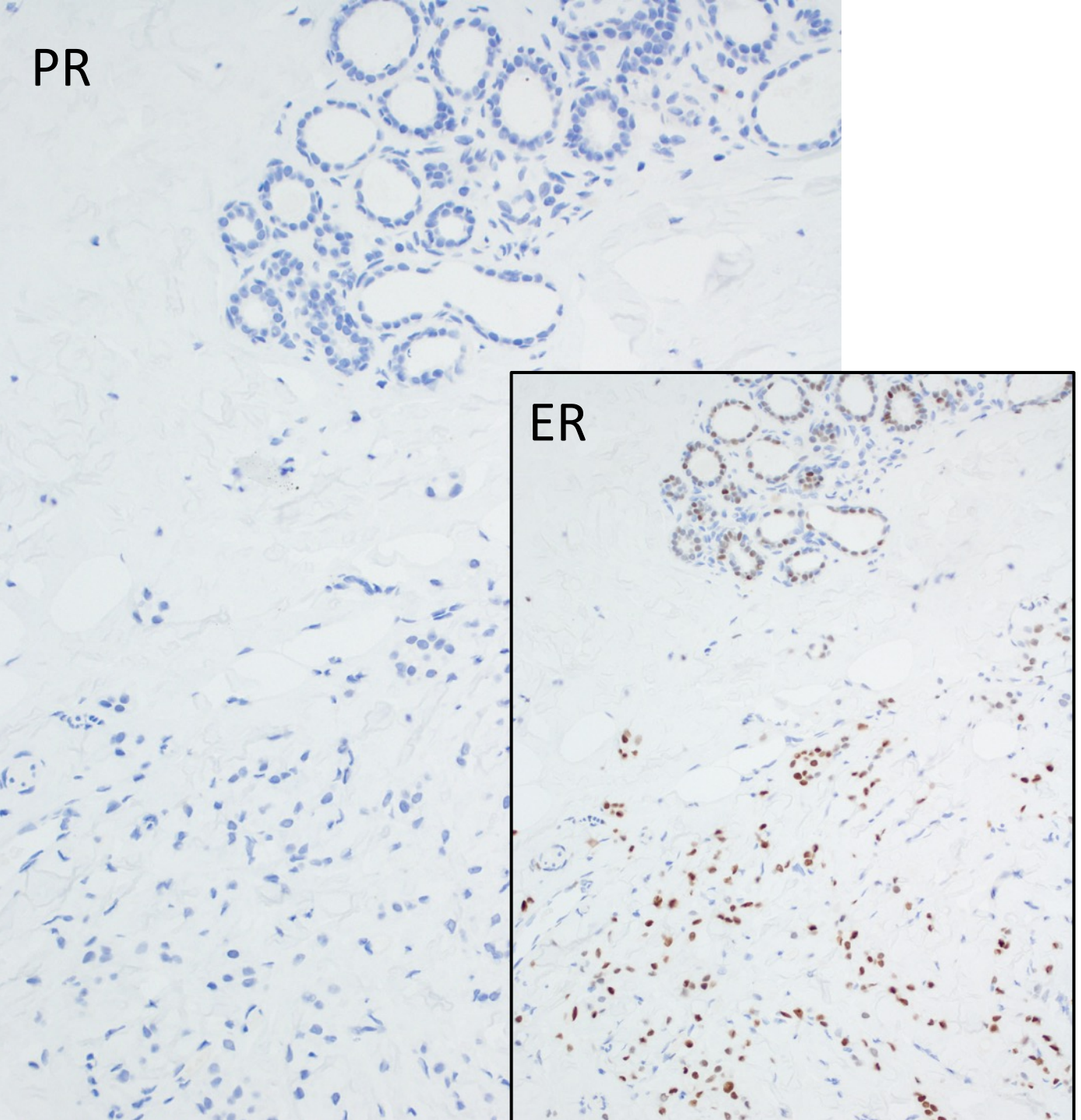


Troubleshooting

PR stain: ILC with normal breast

What happened?

Next step?



Troubleshooting

PR stain: ILC with normal breast

What happened?

Next step?

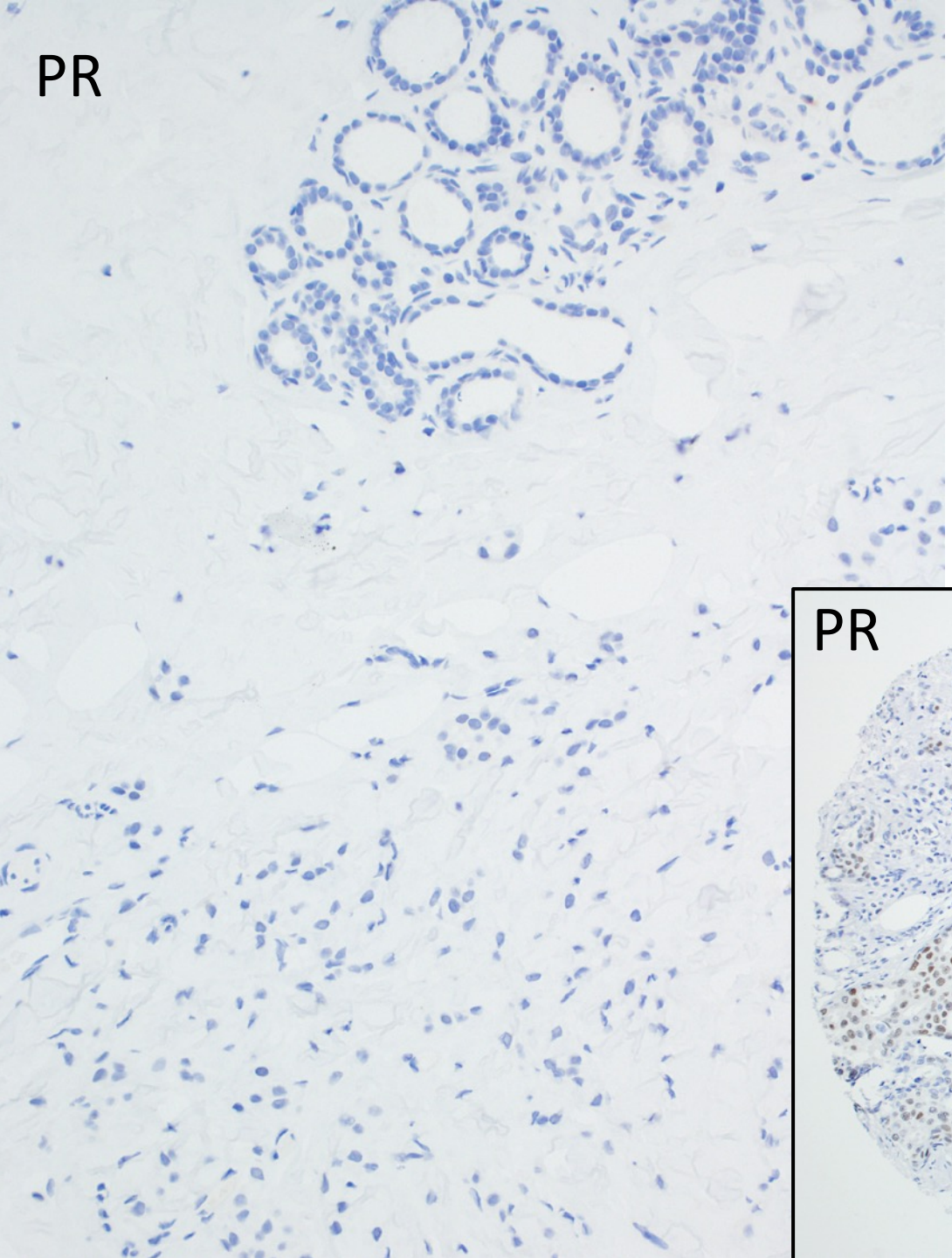
→Check ischemia/fixation

Ischemia: 1 hr

Fixation: 10 hr 10% NB formalin

ER worked!?

Allison. *Arch Pathol Lab Med*. 2020;144:545-563



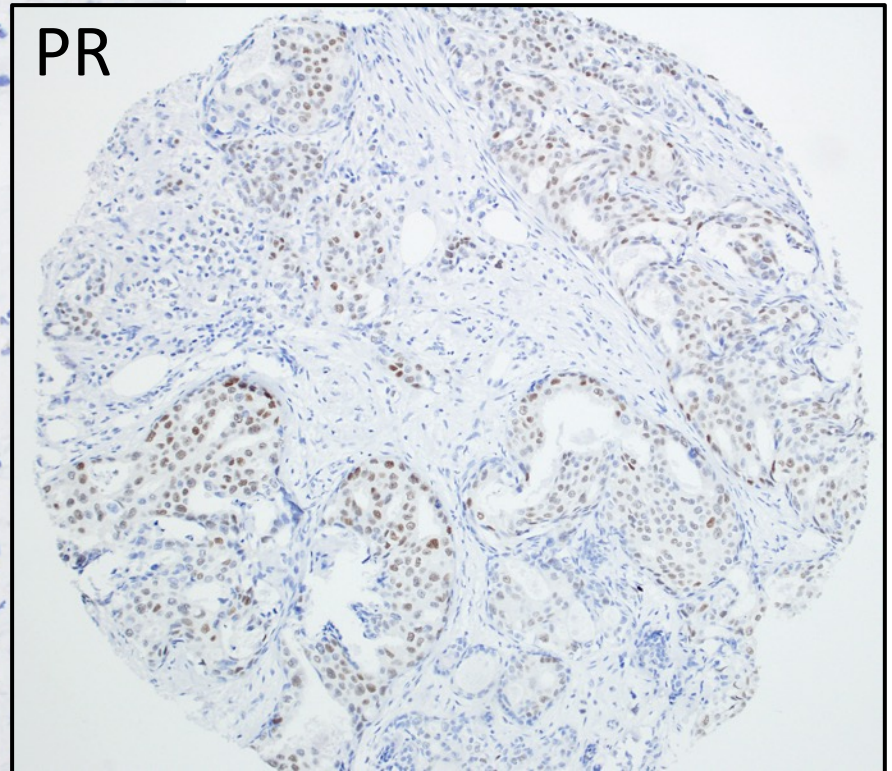
Troubleshooting

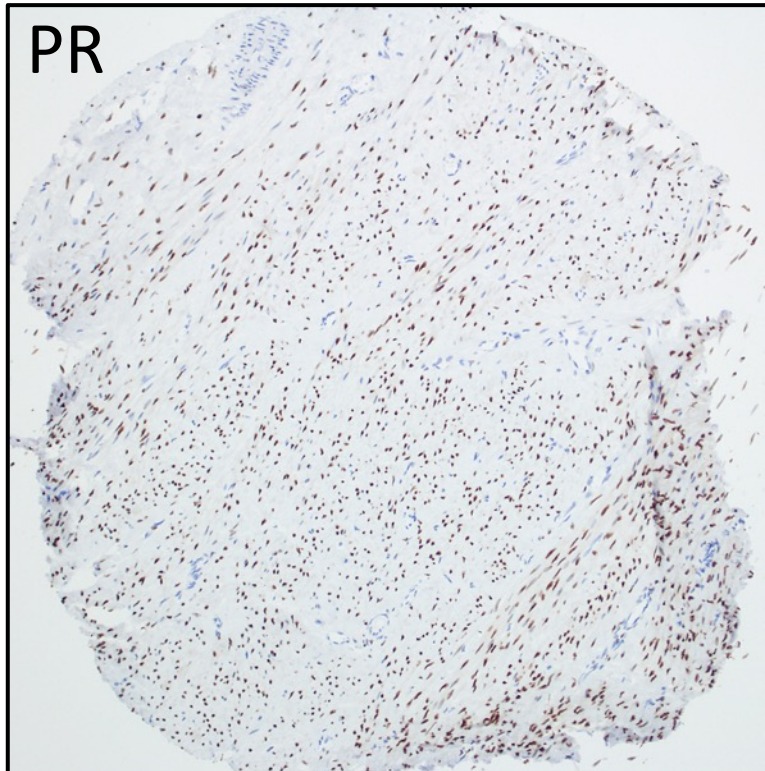
PR stain: ILC with normal breast

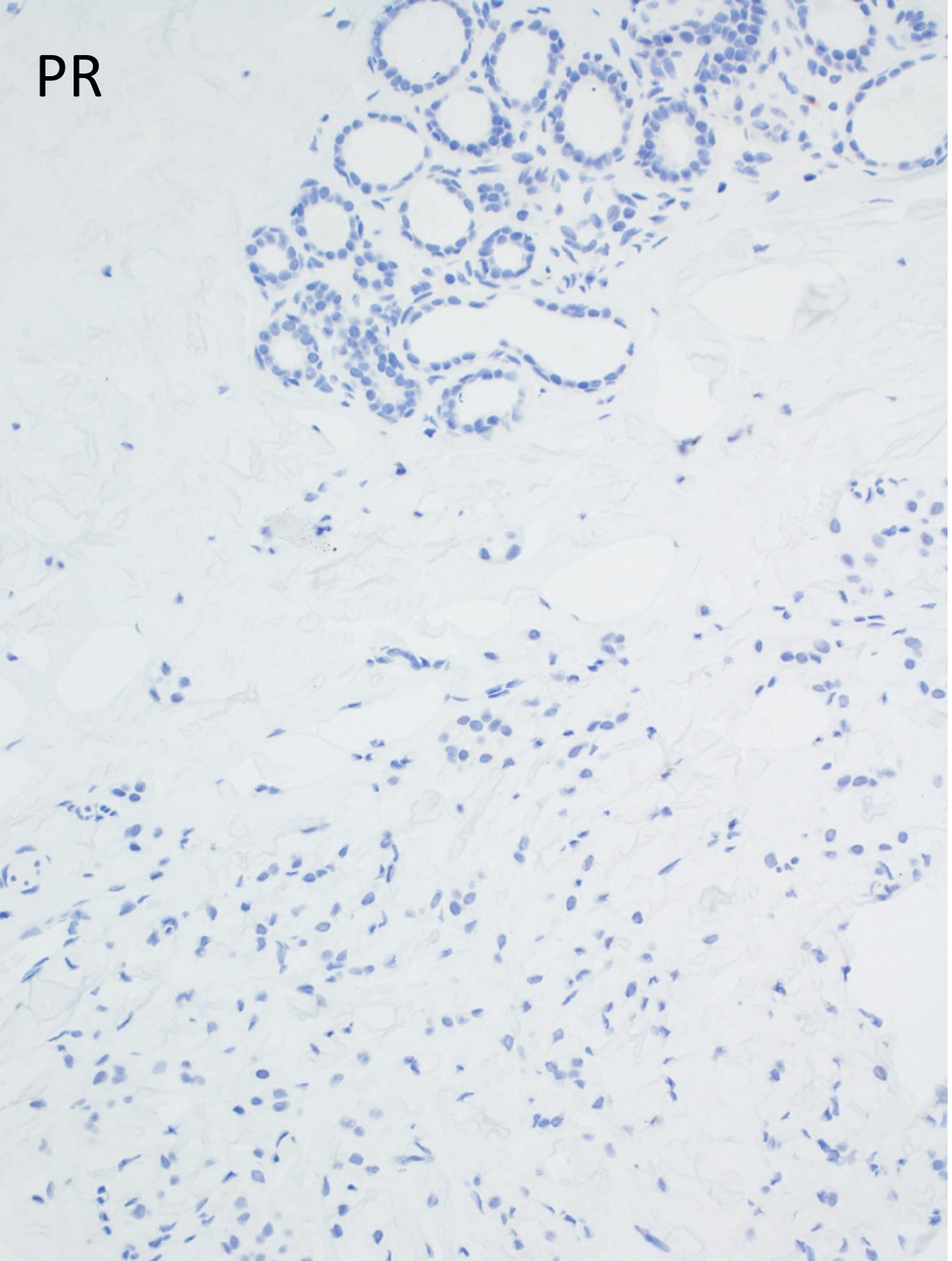
What happened?

Next step?

→Check onslide external control







Troubleshooting

PR stain: ILC with normal breast

What happened?

Next step?

→Correlate with clinical history

Prior core ER+++/PR+++

Neoadjuvant letrozole Rx

→Repeat stain? Same

Now what?

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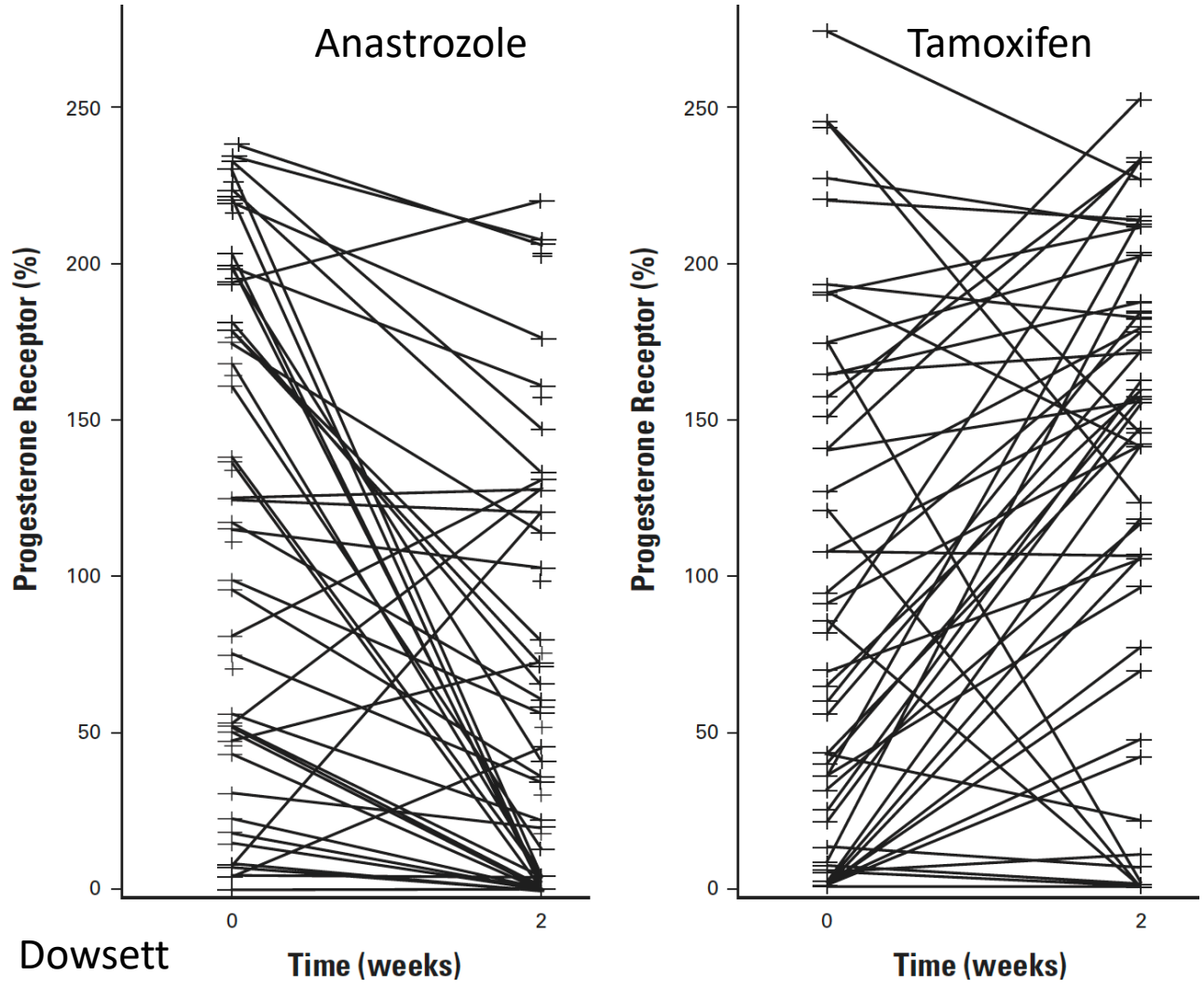
ER PR IHC with endocrine therapy



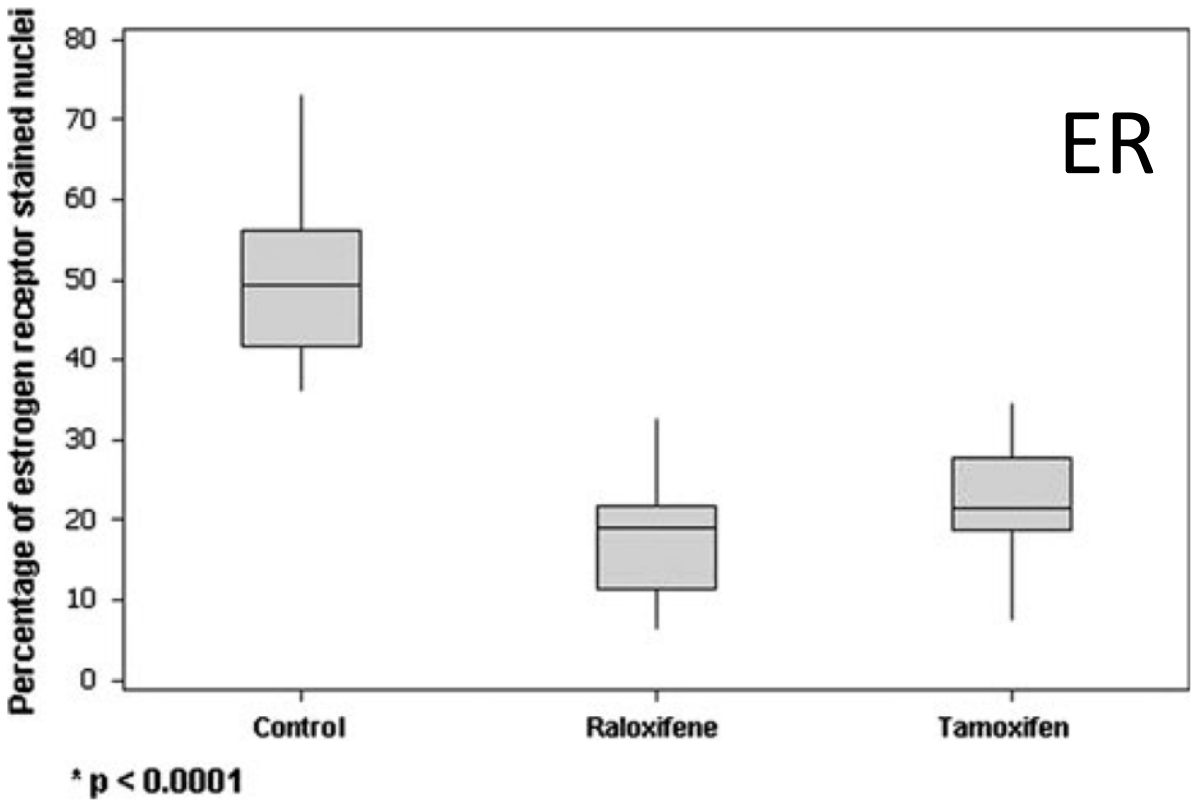
Dowsett

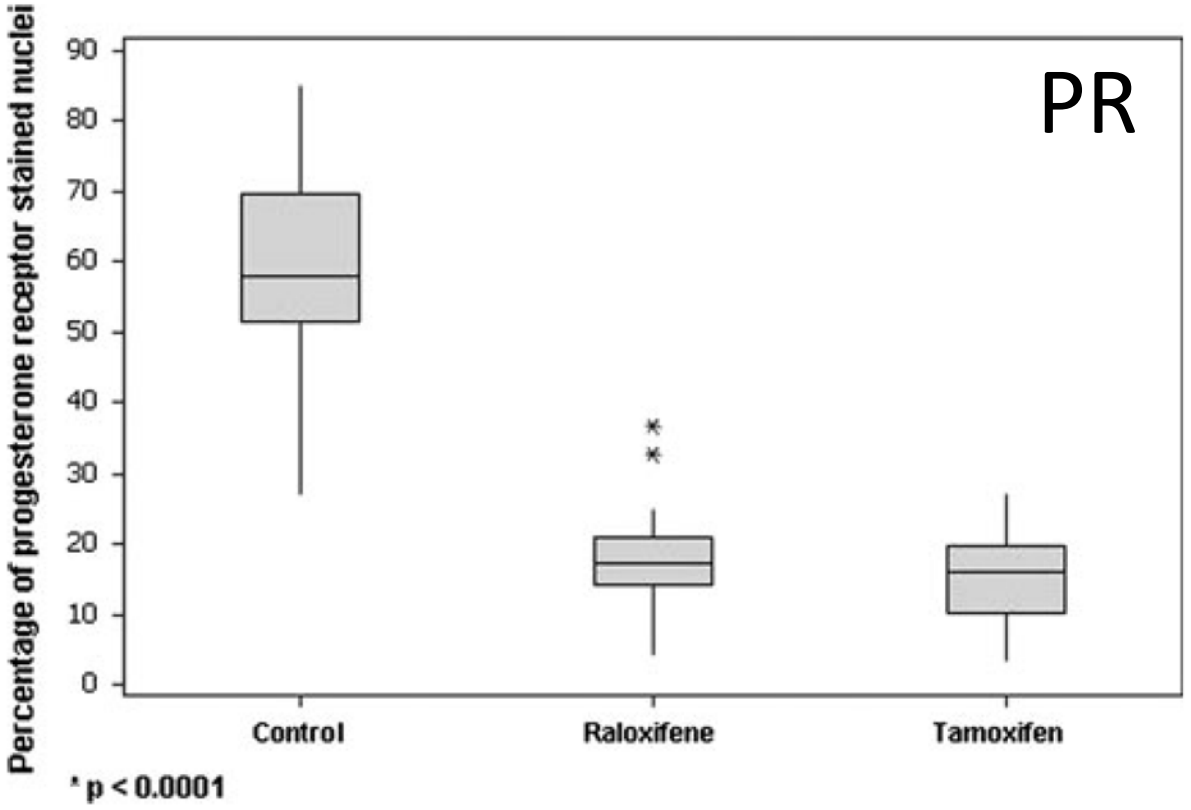
- Profound decrease in PR with Aromatase inhibitor
  - Tumor and normal
  - Letrozole, anastrozole, exemestane
- Effects of tamoxifen may vary over time and differ in tumor/normal

	ER	PR
Tam	Partial decrease	Variable
AI	Stable	Decrease/neg

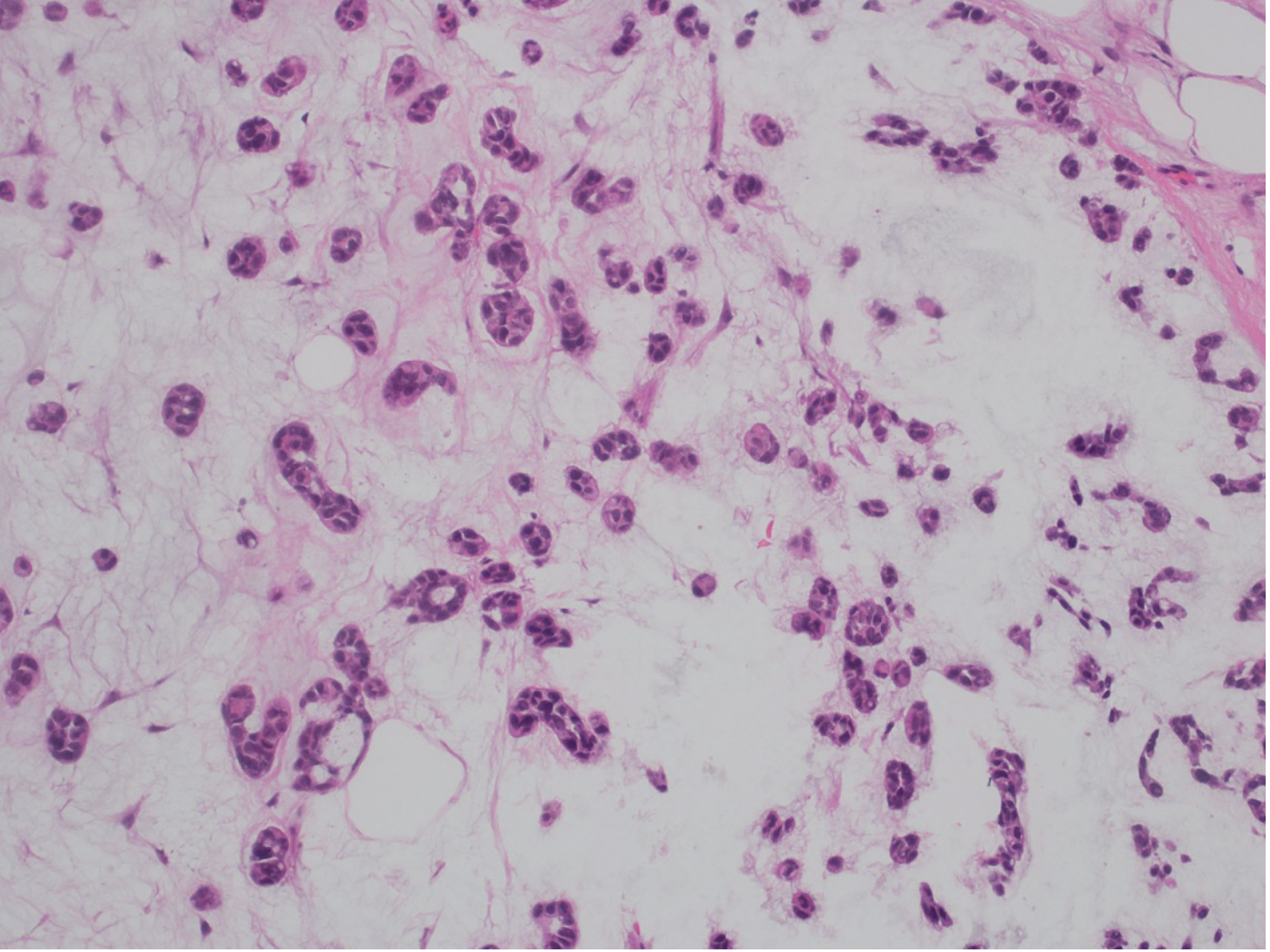
Miller. J Steroid Biochem Mol Biol. 2005;95:83-9
Dowsett. J Clin Oncol 23:2477-2492.
Kurosumi. J Cancer Res Clin Oncol.2008;134:715–22

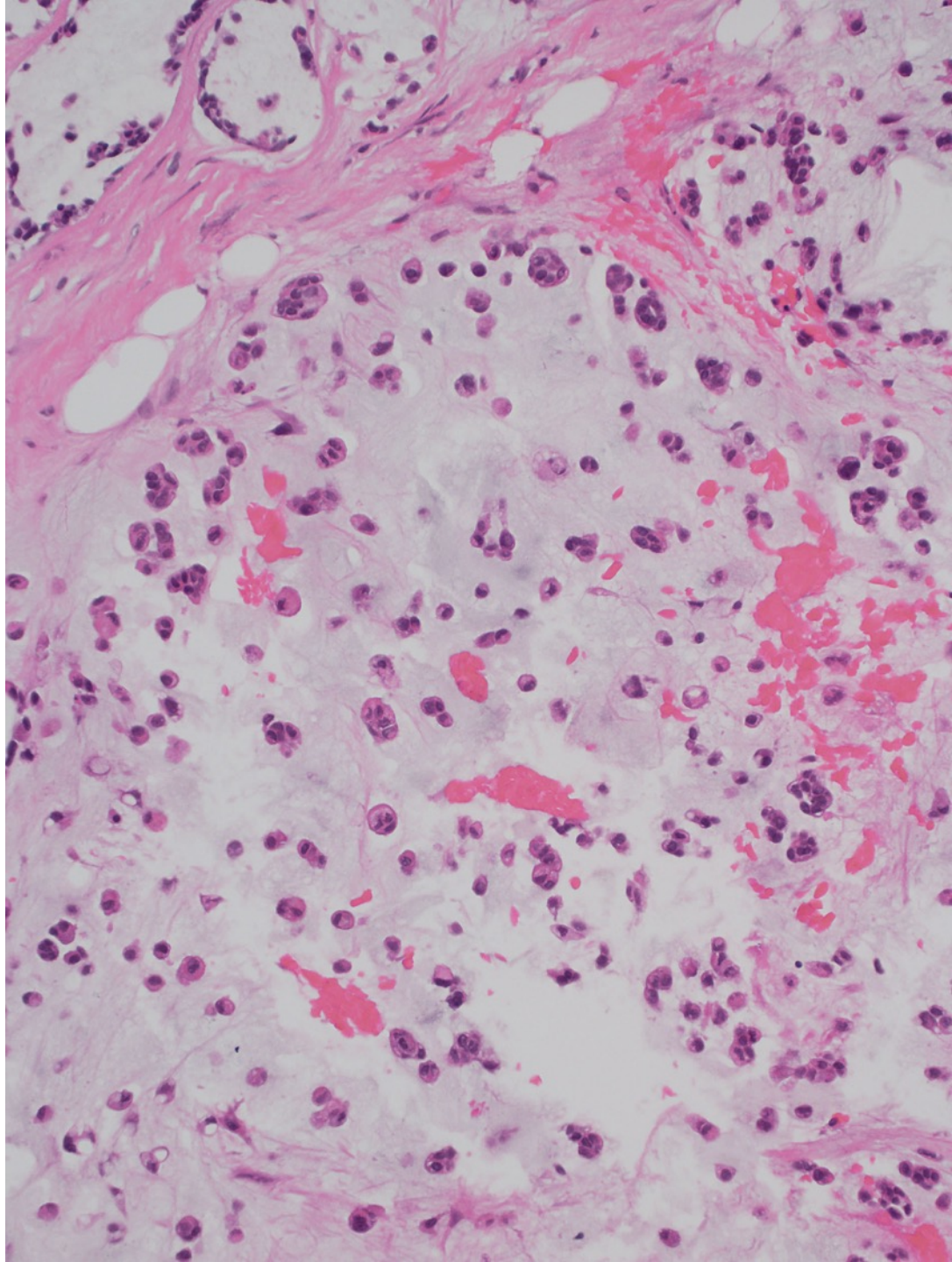
ER PR IHC with endocrine therapy



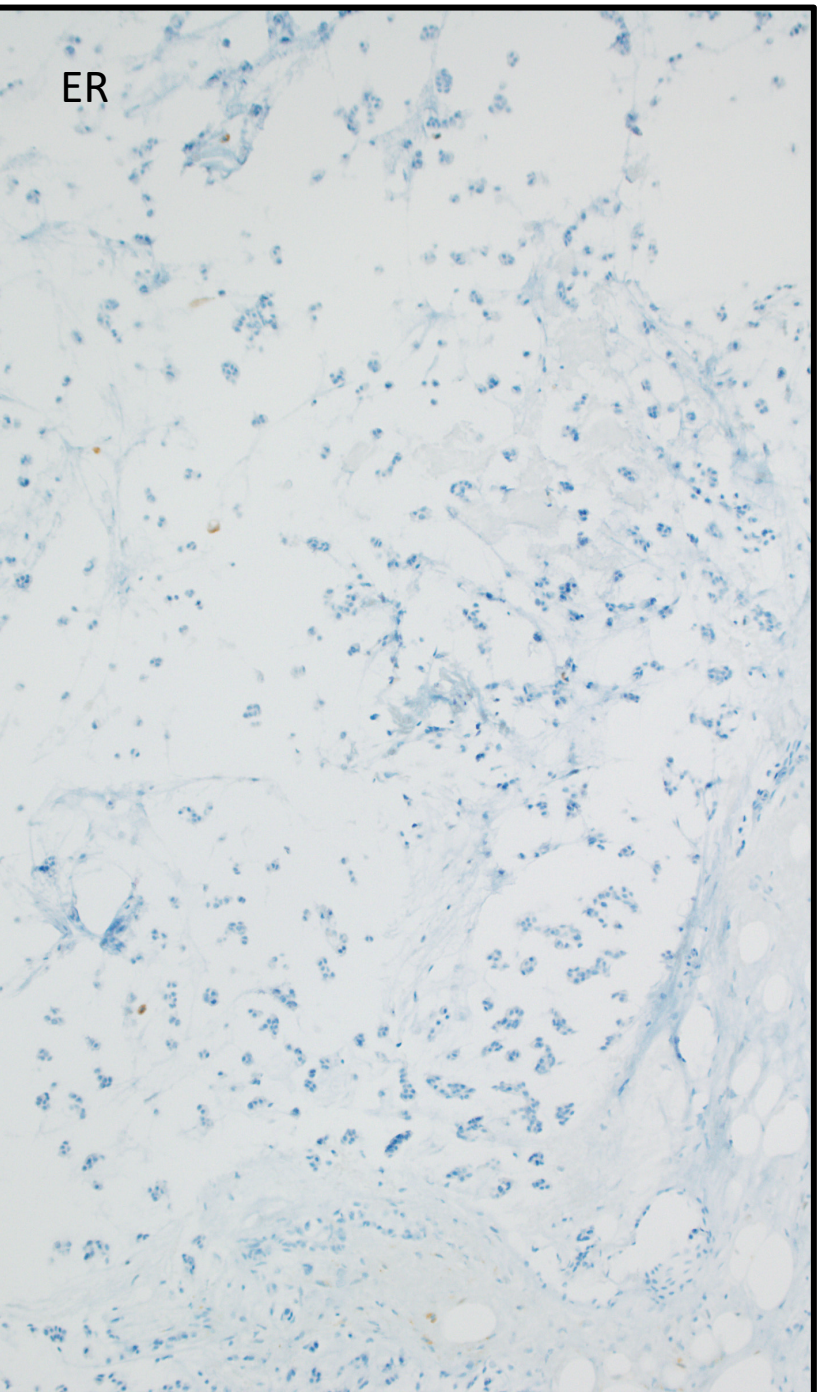


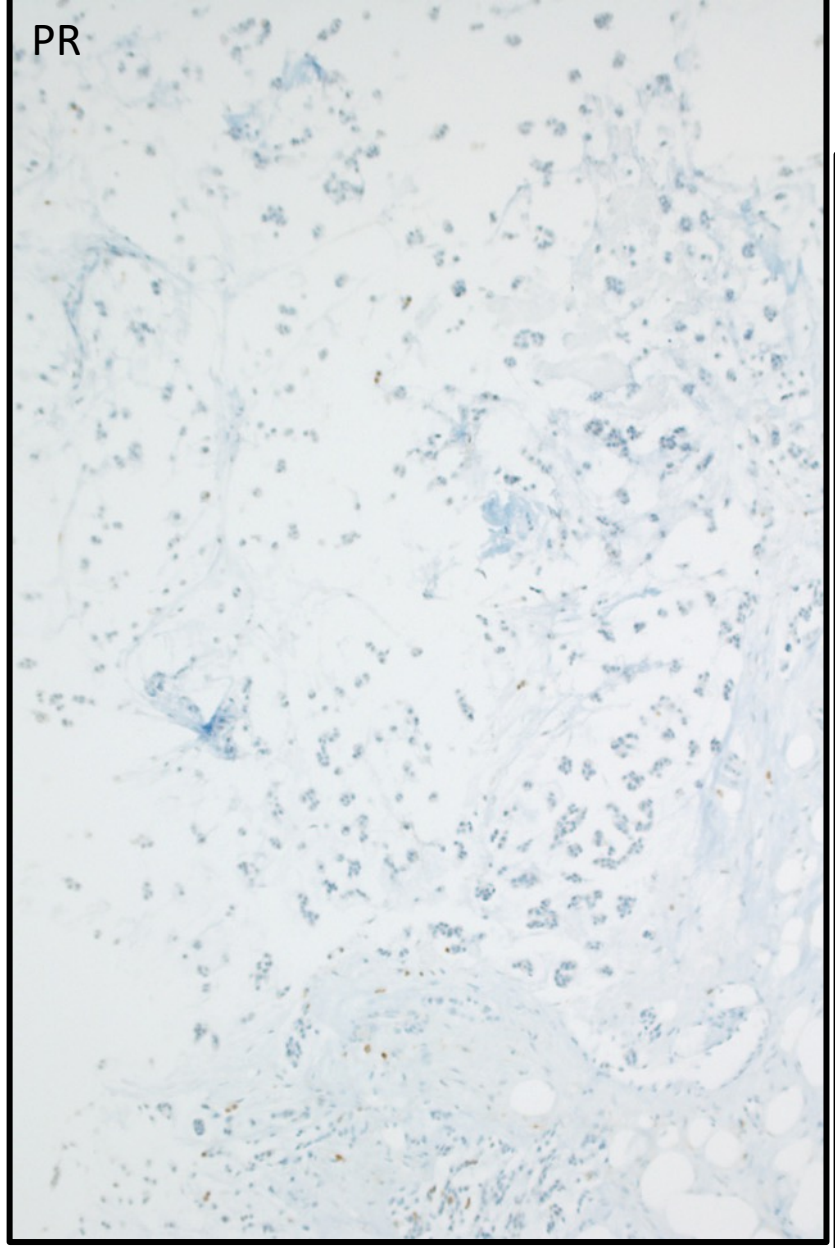
Rosal. Breast Cancer Res Treat 2011;125:797–801
Normal breast, 20 day course

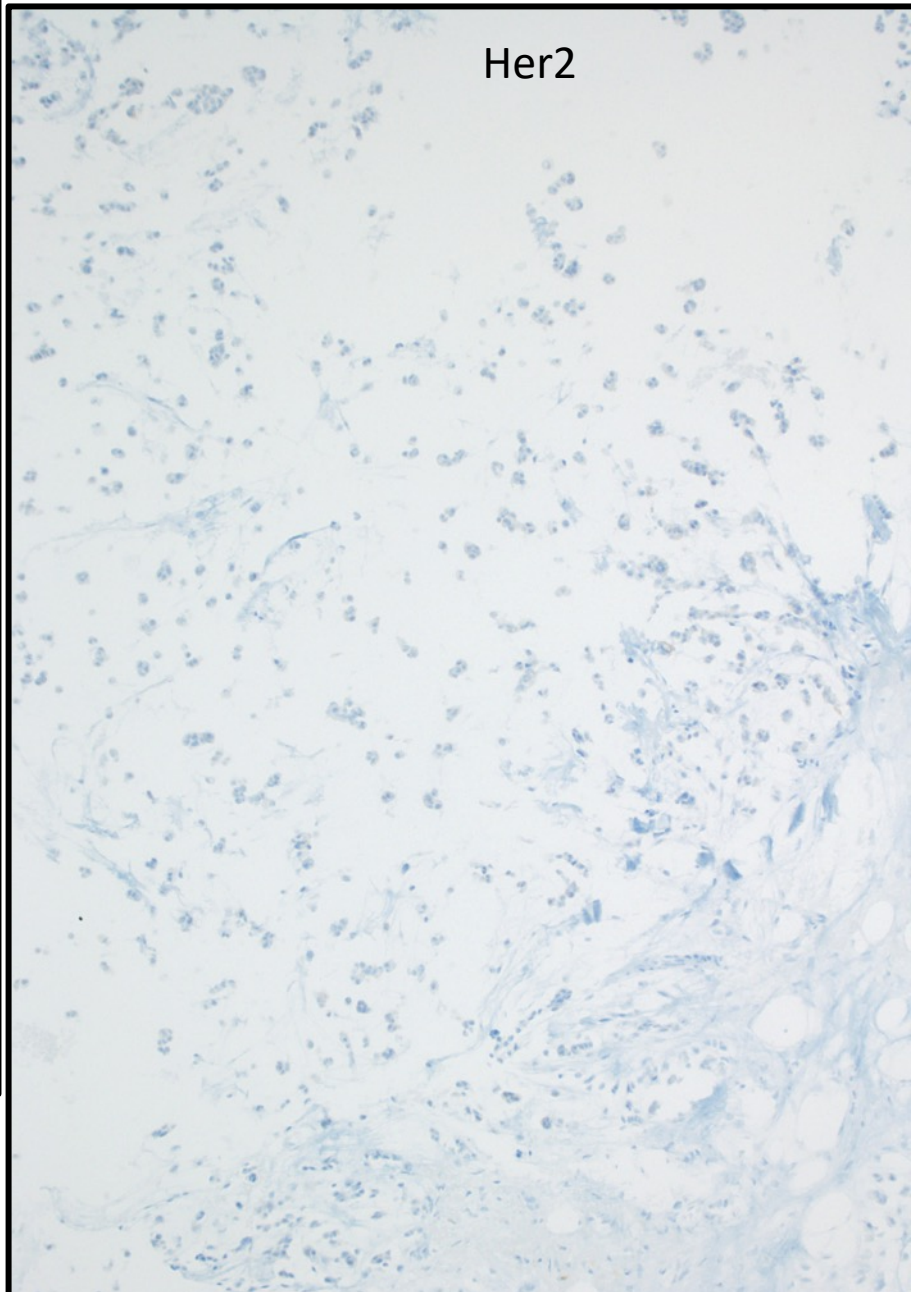




Mucinous carcinoma; 1.1 cm

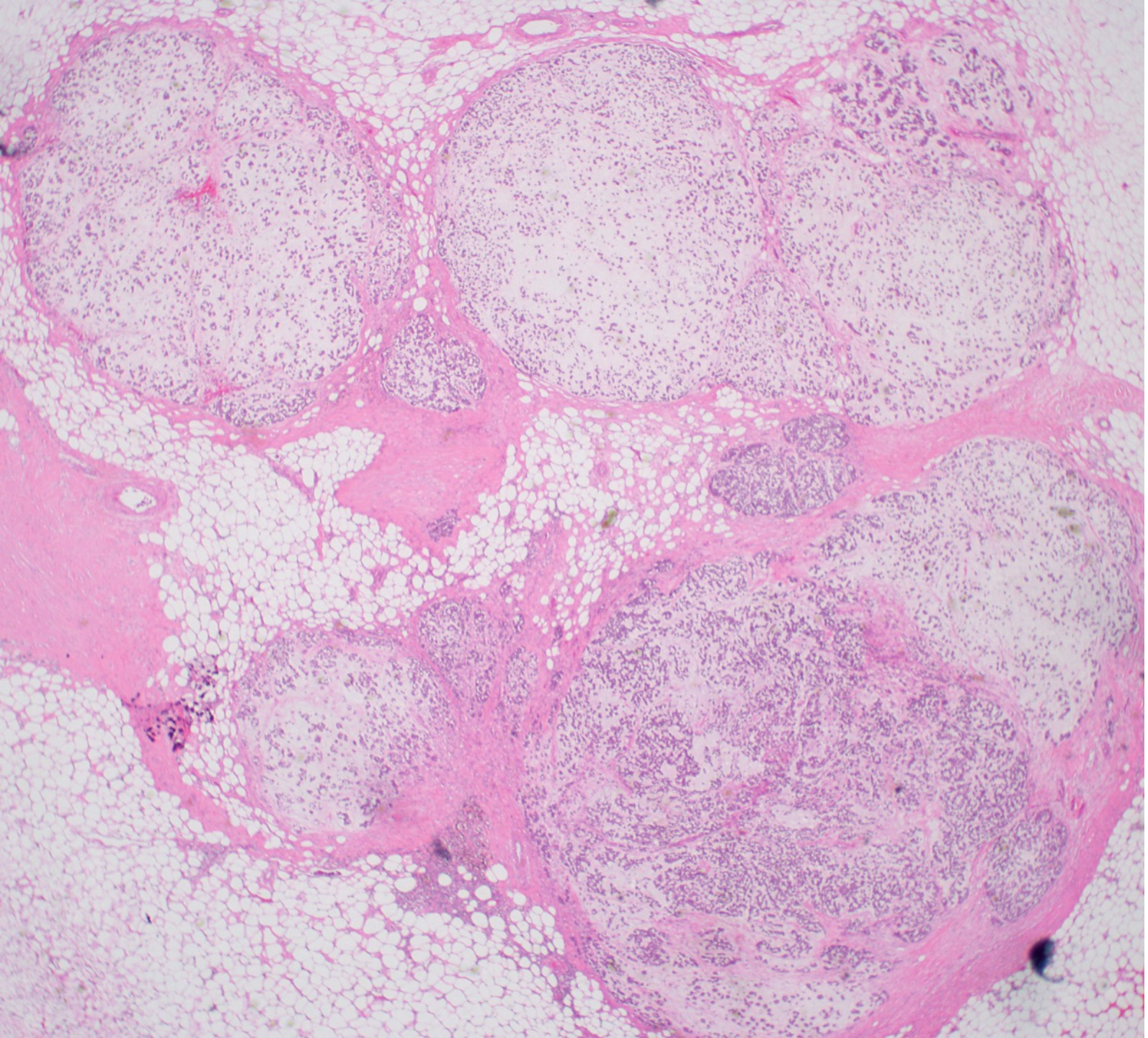






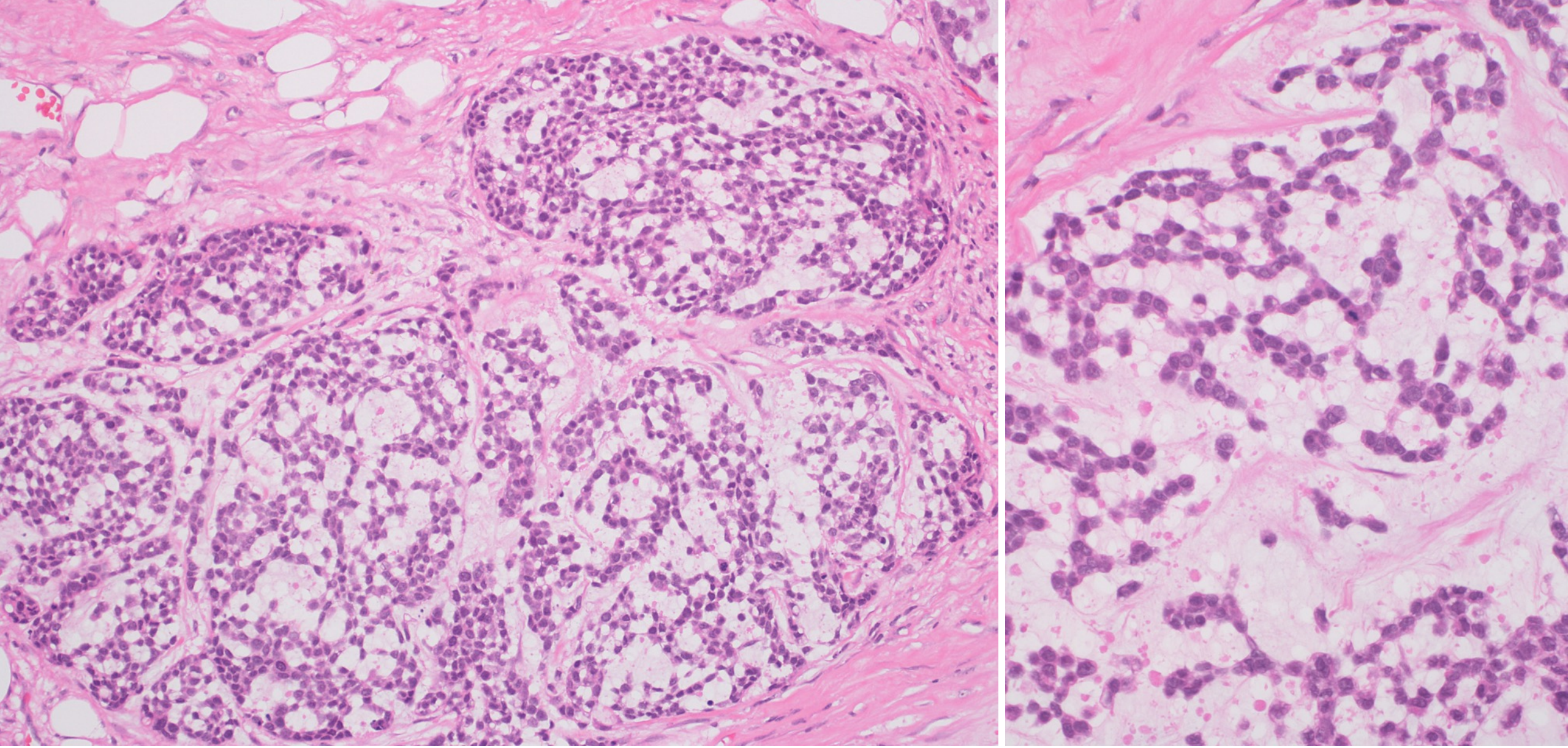
Internal controls +

Now what??



Resection

- Multinodular
- Hypo/Hypercellular areas
- ER-
- PR-
- Her2-



LG metaplastic “matrix producing” ddx: of salivary type/myoepithelial; has recurred

ER: histologic concordance

Expected ER+++

- Low grade IDC
- Classic ILC
- Mucinous
- Tubular
- Cribriform
- Low grade DCIS
- Encapsulated/solid papillary

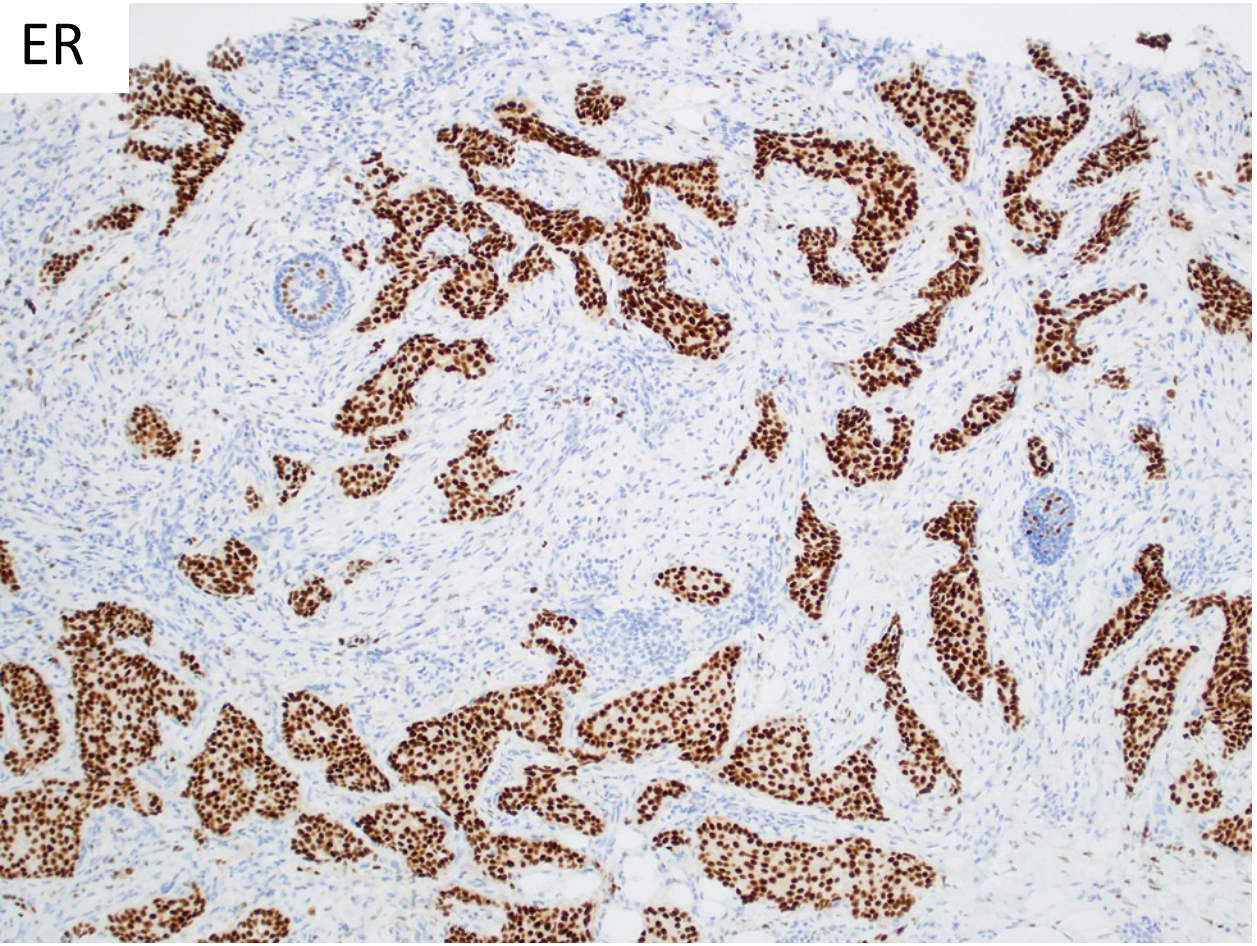
Low grade, but expected ER-

- Adenoid cystic
- Secretory
- Metaplastic
  - Low-grade adenosquamous
  - Well-differentiated squamous
  - Low grade fibromatosis-like
- Low-grade apocrine
- Microglandular adenosis (not carcinoma!)
- Metastasis (Gyn ER+)

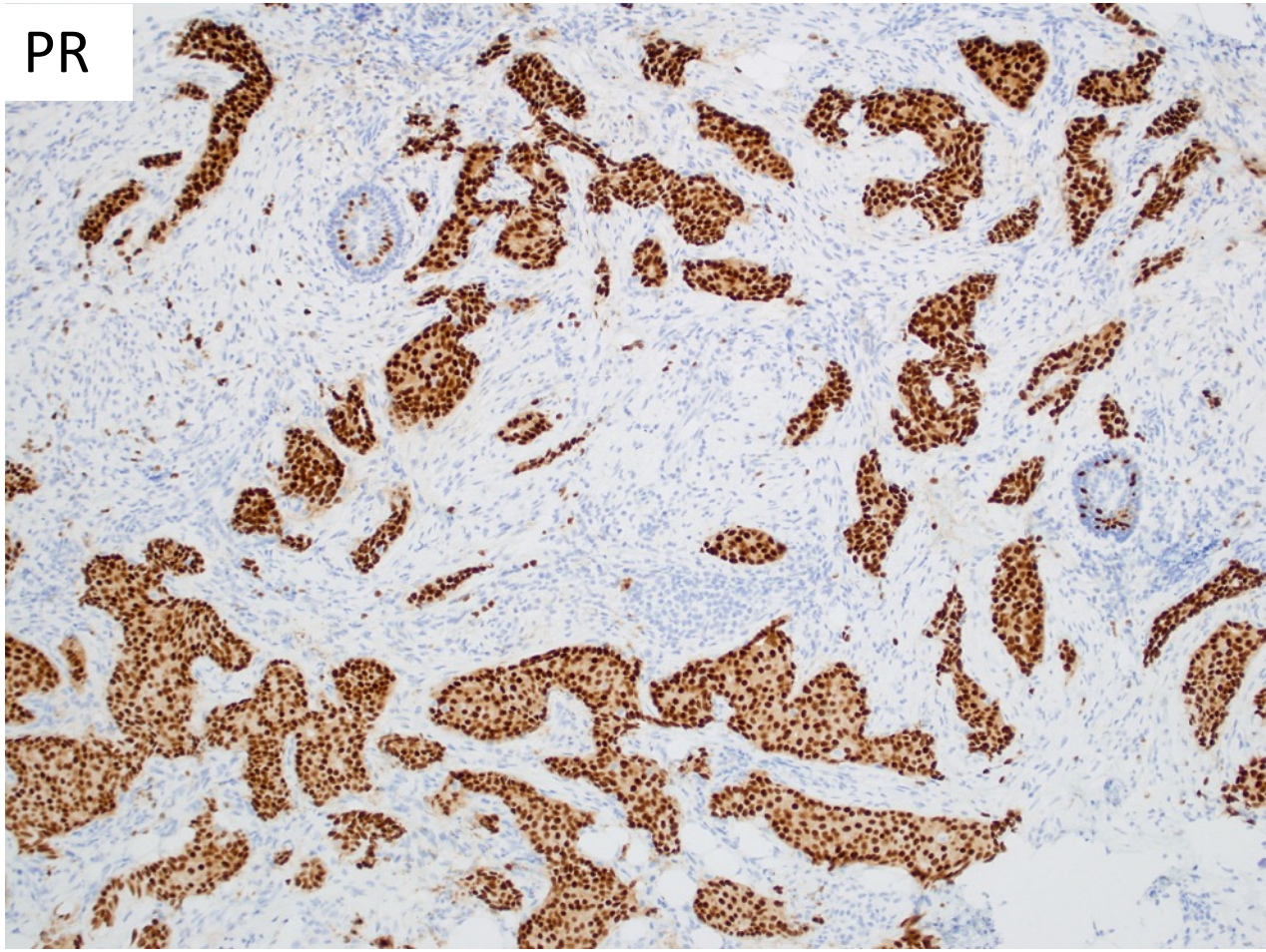
Allison KH. Surg Pathol Clin. 2018;1:147-76

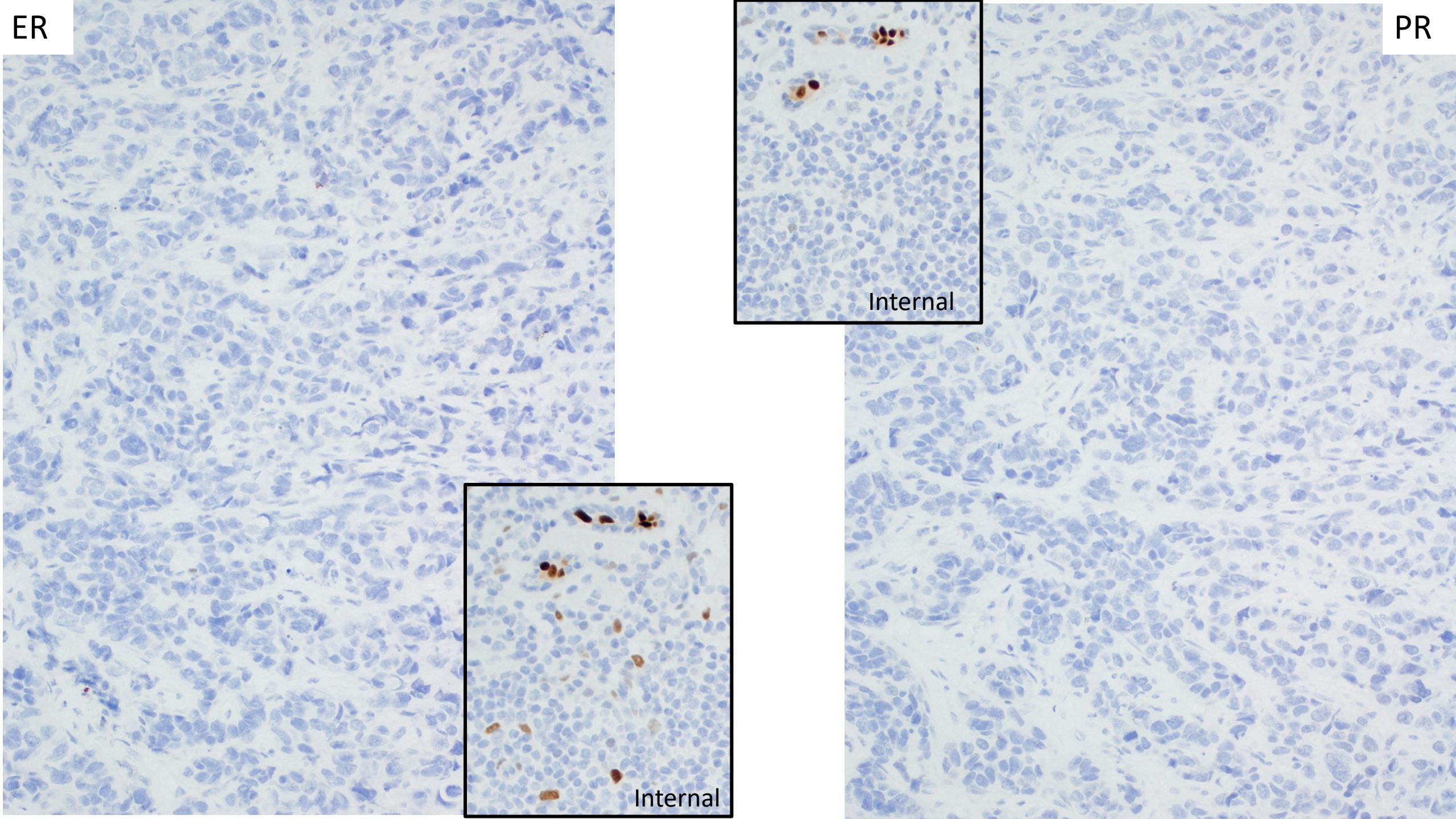
ER & PR in real life

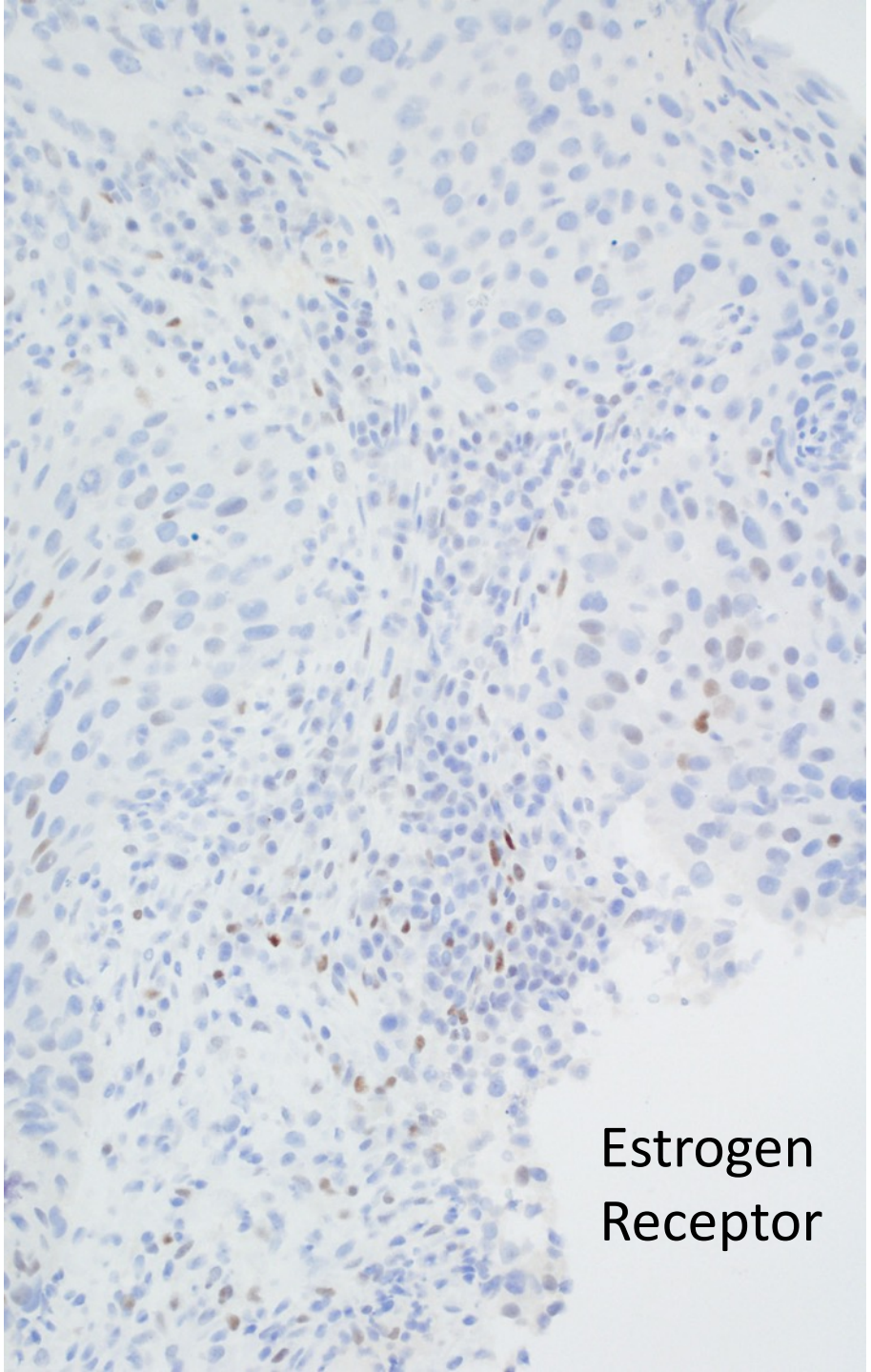
ER



PR



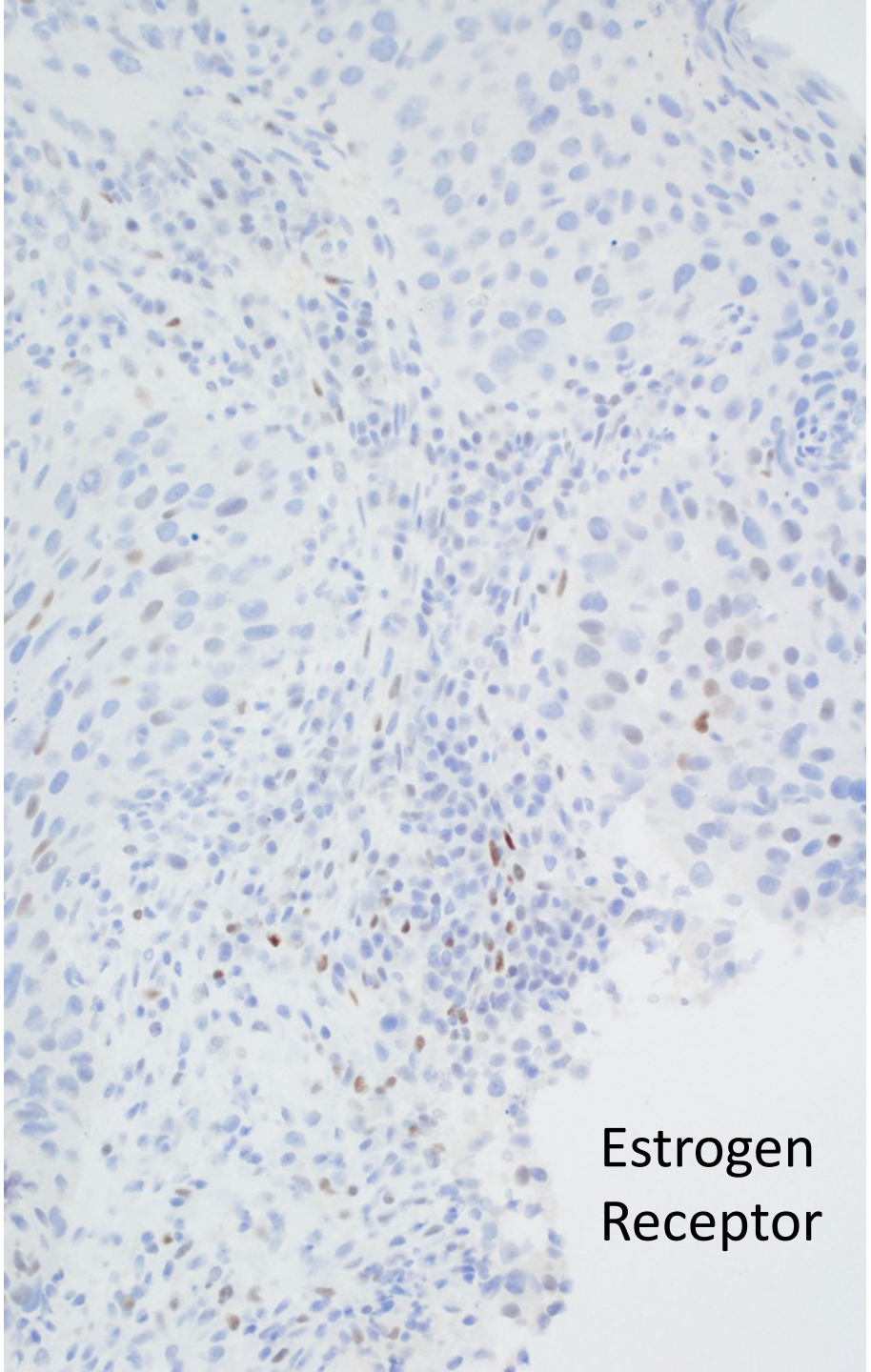

ER
Internal
Internal
PR

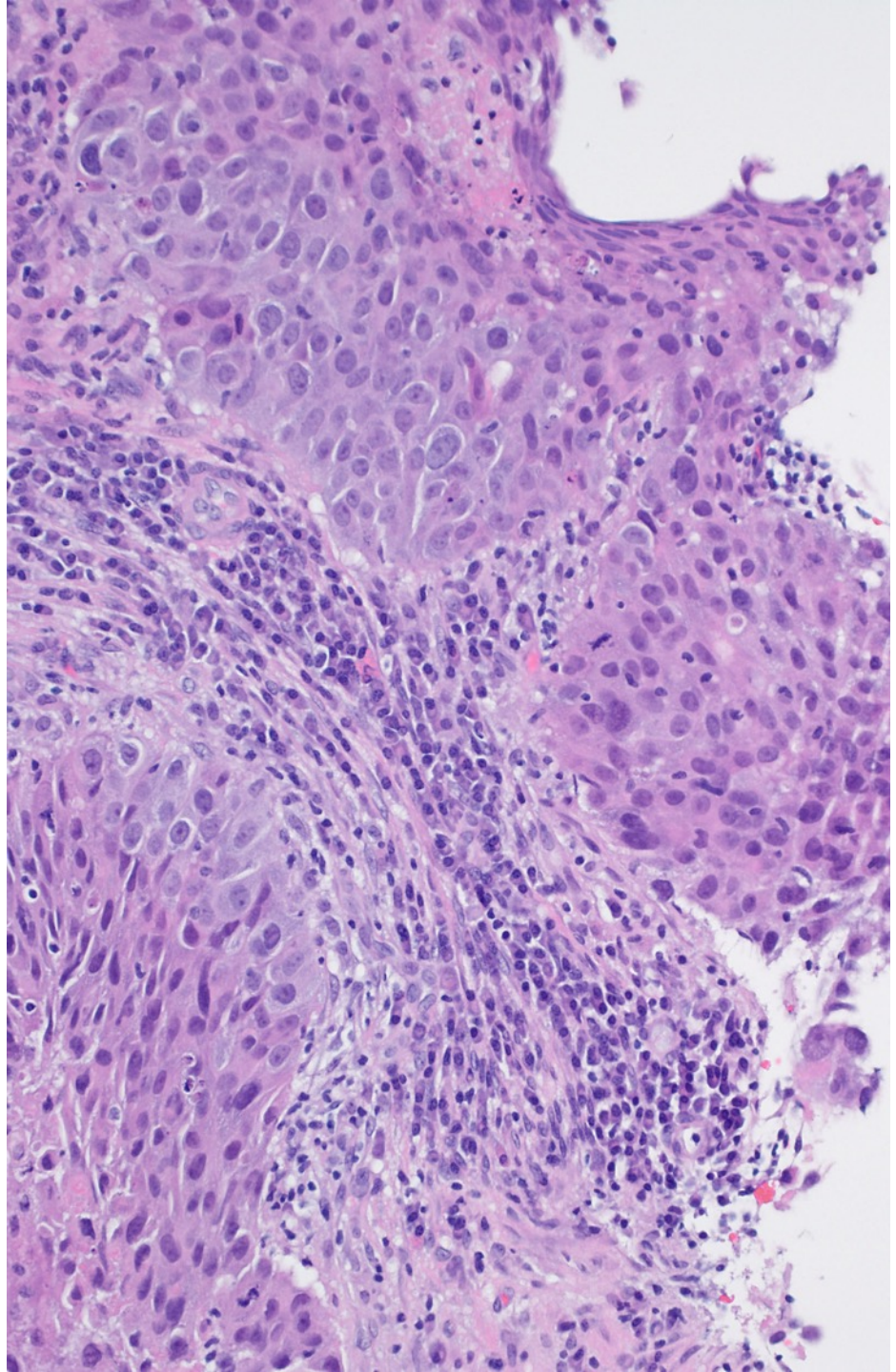


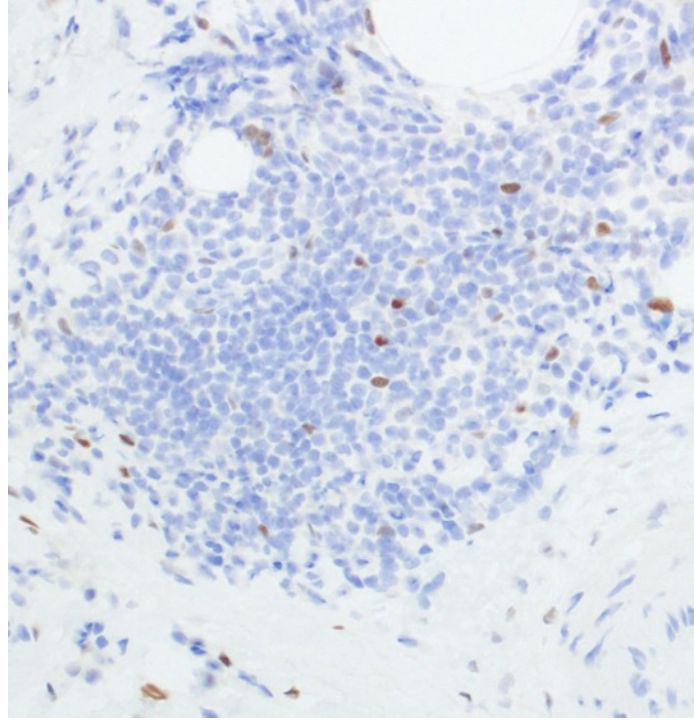
How would you score this ER?

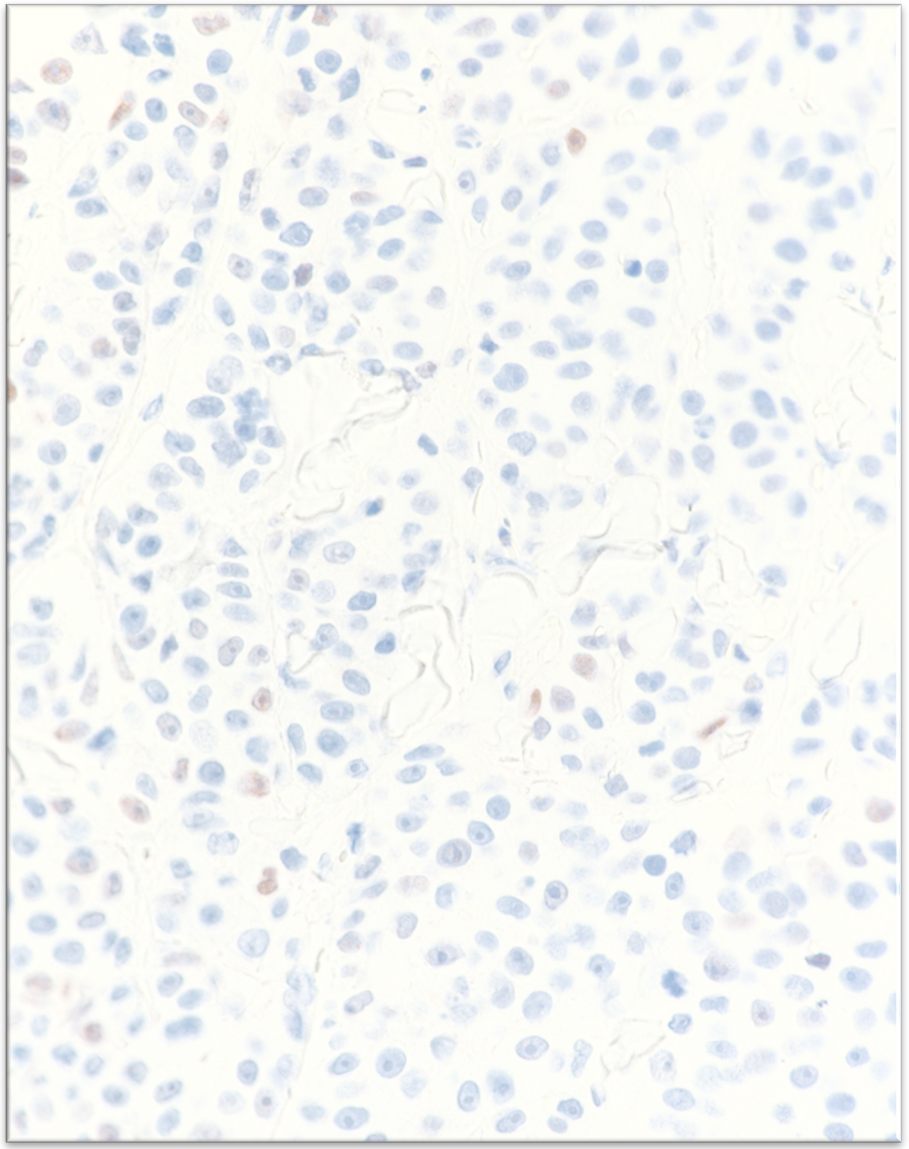
A. Negative (0 to <1%)
B. Low Positive (1-10%, weak)
C. Positive (10-50%, weak)

Allison. *Arch Pathol Lab Med*. 2020;144:545-563


Estrogen
Receptor







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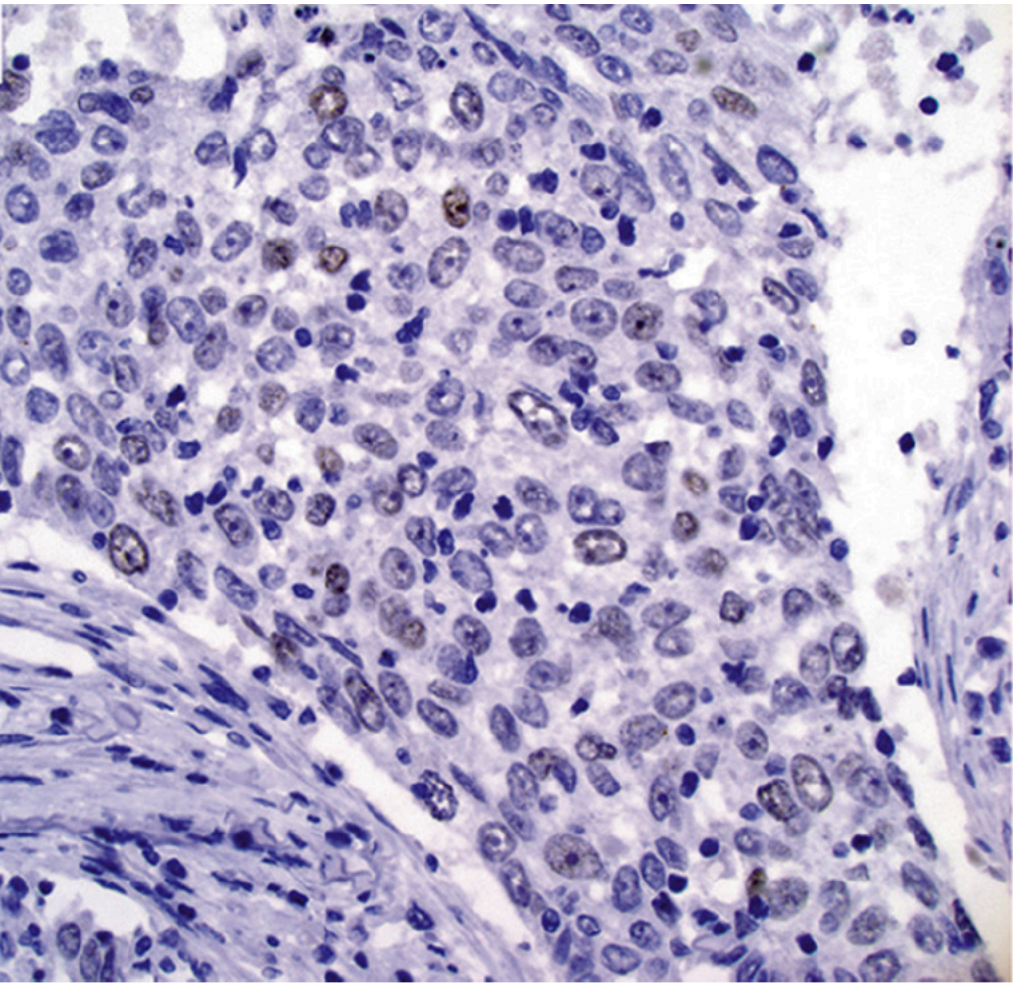
Allison. *Arch Pathol Lab Med*. 2020;144:545-563
Winters C, Allison KH, unpublished



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Allison. *Arch Pathol Lab Med*. 2020;144:545-563
Allison KH. Surg Pathol Clin. 2018;1:147-76

ER PR: consider retest on surgical specimen

- Initial core biopsy result is borderline, insufficient (or very small), equivocal, unusual
- Result discordant with histologic or clinical findings
- Heterogeneity of grade or morphology on surgical sample
- Questionable specimen handling of initial core (long ischemic time, short time in fixative, alterative fixative used)
- Stanford practice, also retest:
  - Core results from outside lab
  - Post-neoadjuvant chemotherapy

Allison KH. Surg Pathol Clin. 2018;1:147-76

ER PR: recap

- Perform and report hormone receptor studies as per ASCO/CAP guidelines
  - Attention to pre-analytic (fixation/ischemic time)
  - Attention to internal and external controls (esp. on-slide)
  - Positive threshold: >=1% of tumor nuclei
    - **New Low Positive category: 1-10+% tumor nuclei, and lab SOP's**
    - May behave more similar to ER-negative; clinicopathologic correlation needed
  - Report % positive nuclei and intensity
  - Proficiency testing, pathologist concordance, benchmark data
  - Test validation

Allison KH. Surg Pathol Clin. 2018;1:147-76

END