

Immunohistochemical classification of breast tumours

Workshop in Diagnostic Immunohistochemistry

September 19th 2016



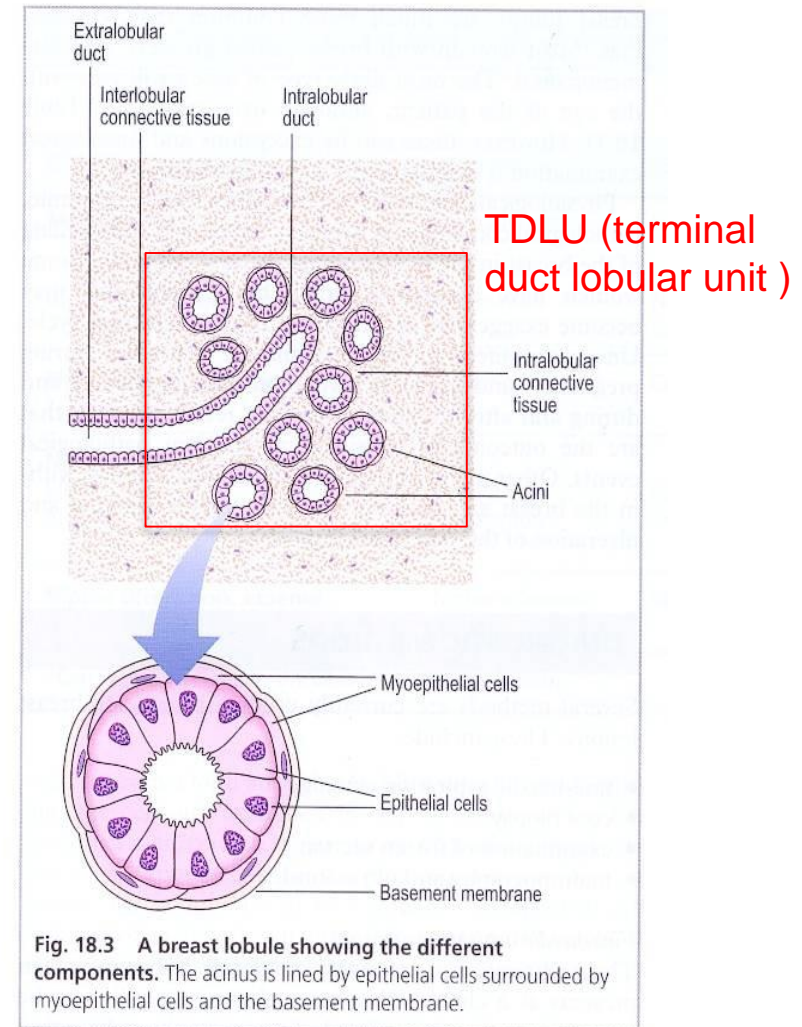
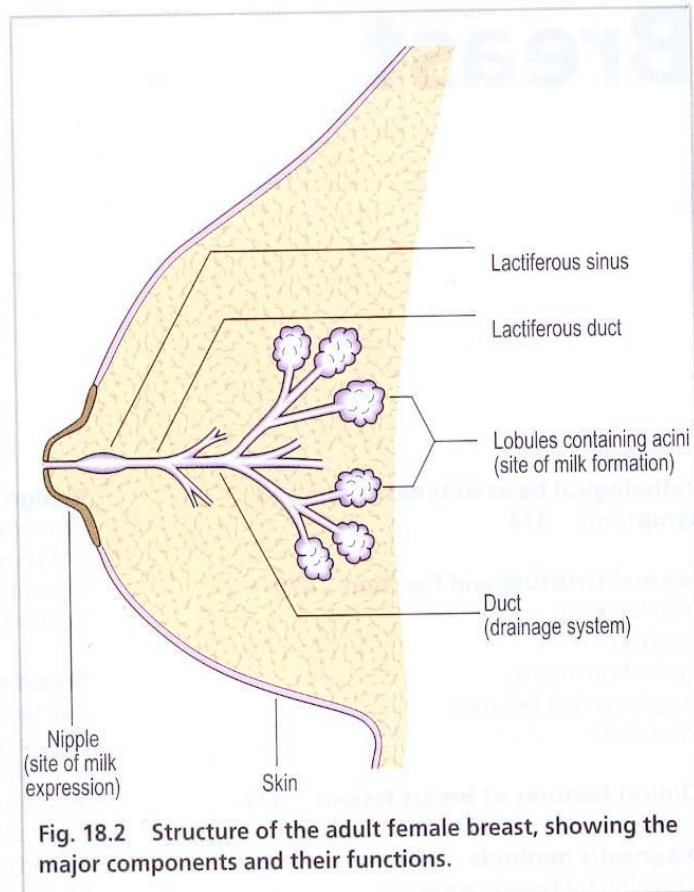
Anne-Vibeke Lænkholm
Department of Surgical Pathology,
Zealand University Hospital,
Slagelse
Denmark

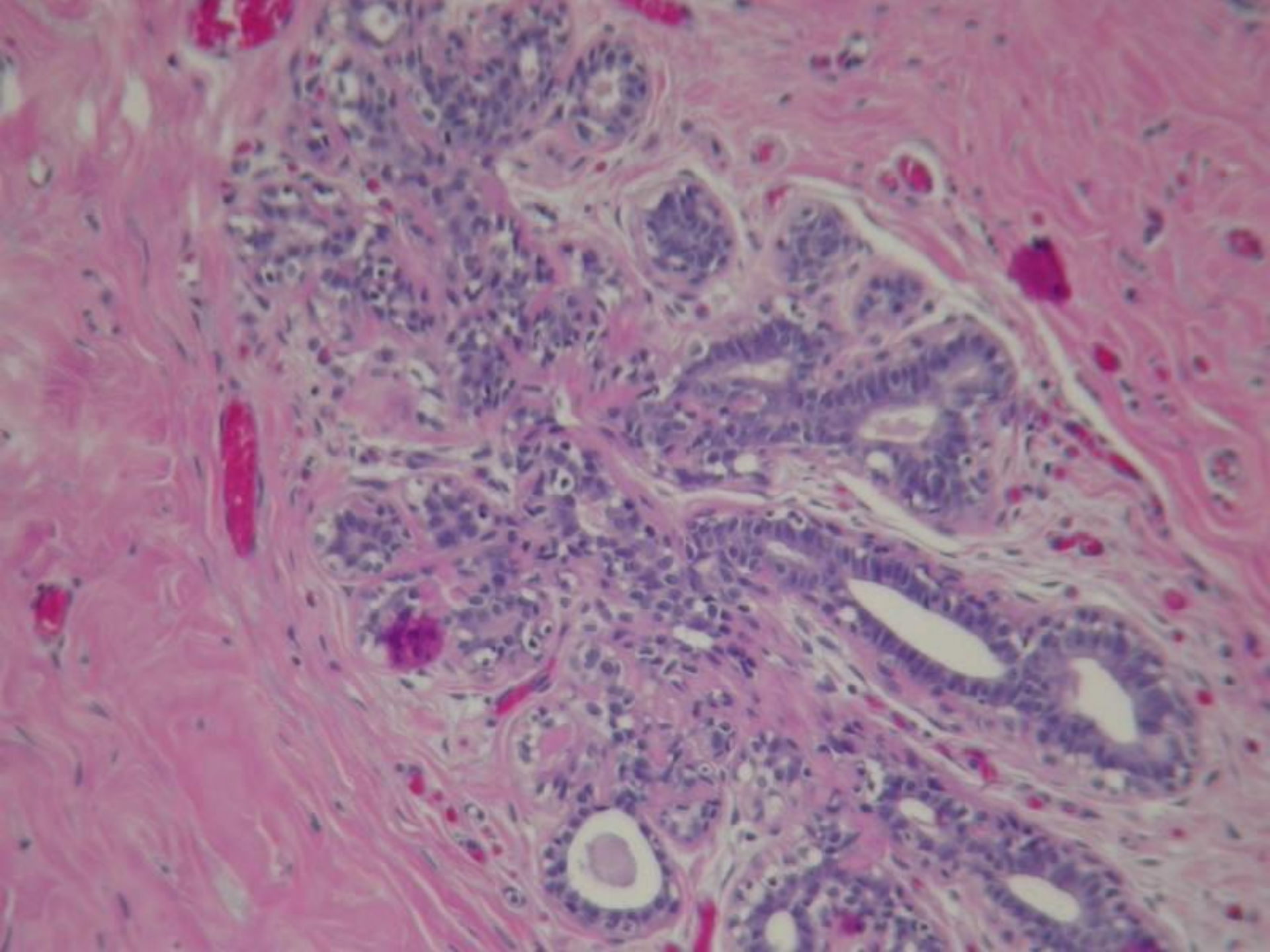


Agenda

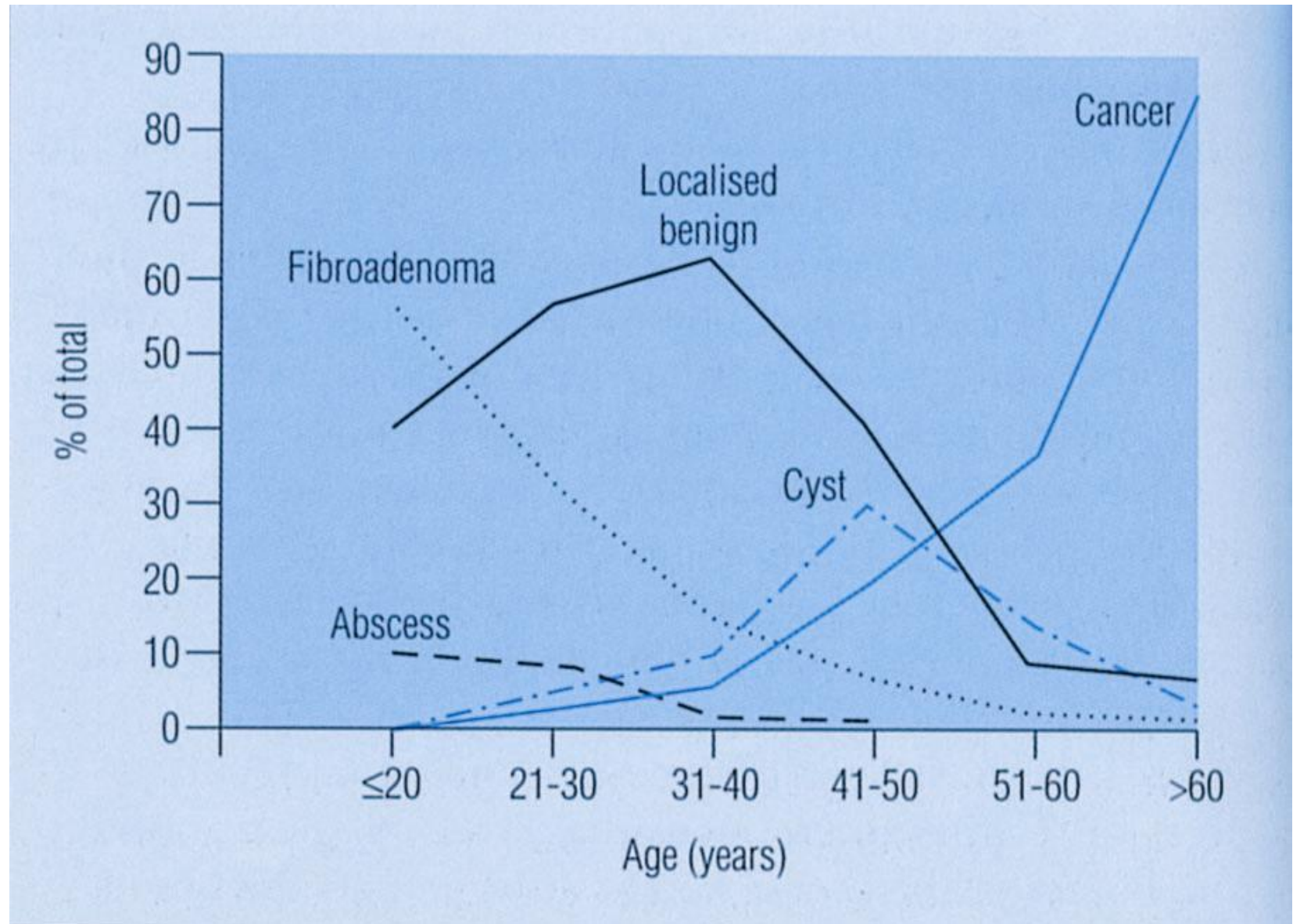
- Immunohistochemical biomarkers for
 - Diagnostics
 - Benign Hyperplasia and Atypical Ductal Hyperplasia
 - Ductal Carcinoma in Situ and Lobular Carcinoma in Situ
 - Carcinoma In Situ and Invasive Carcinoma
 - Invasive Carcinoma
 - Histological subtypes
 - Prediction/Prognosis
 - Estrogen Receptor
 - Progesteron Receptor
 - HER2
 - Ki67
 - Intrinsic subtype classification by surrogate biomarkers

Normal Breast



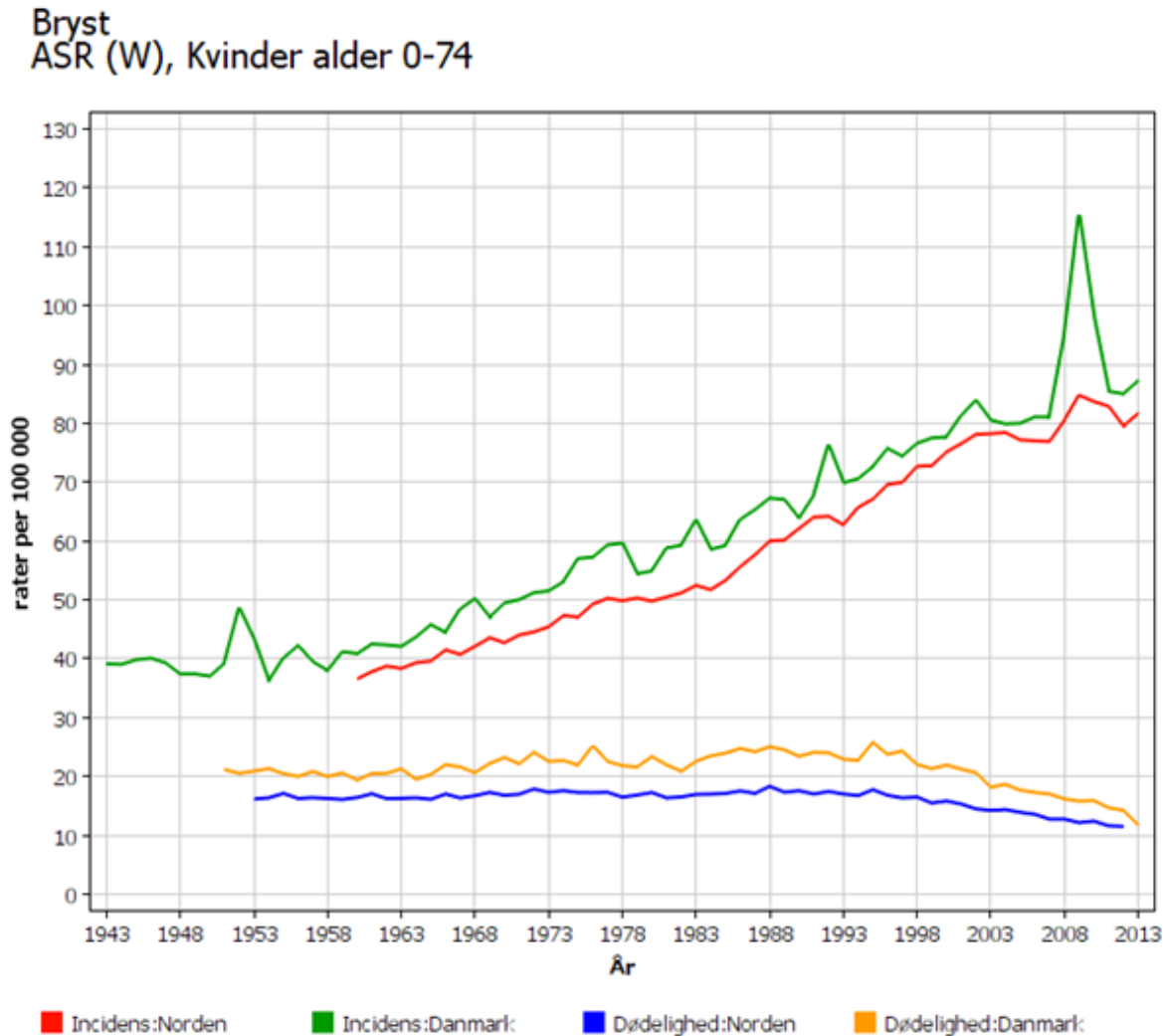


Breast tumours according to age



Breast cancer: Incidence and mortality

Denmark



Diagnostic Immunohistochemical Markers

Epithelial markers

- CK8,18,19 (epithelium)
- Cytokeratin 7

Breast

- E- Cadherin
- Mammaglobin
- GCDFP15
- GATA-3
- Androgen Receptor
- Nestin

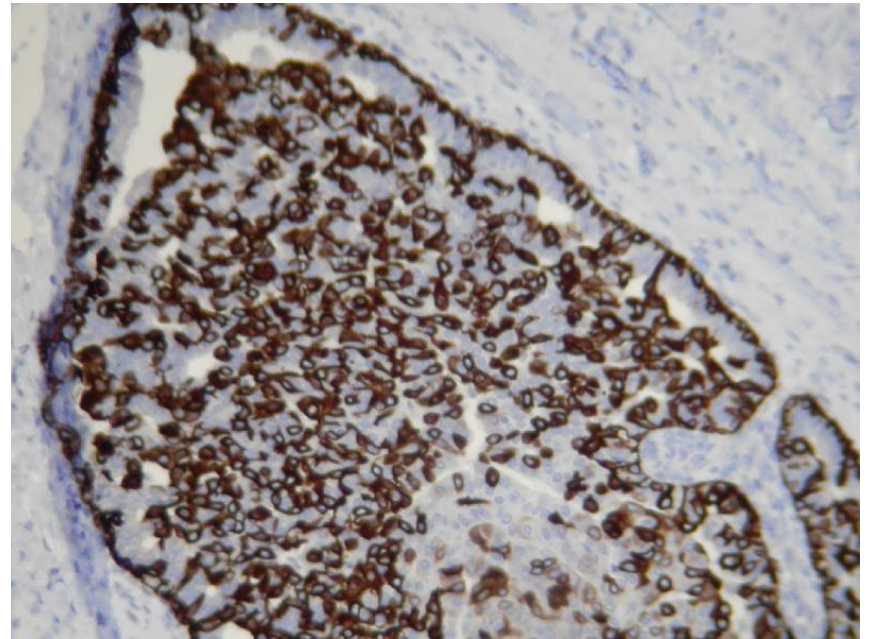
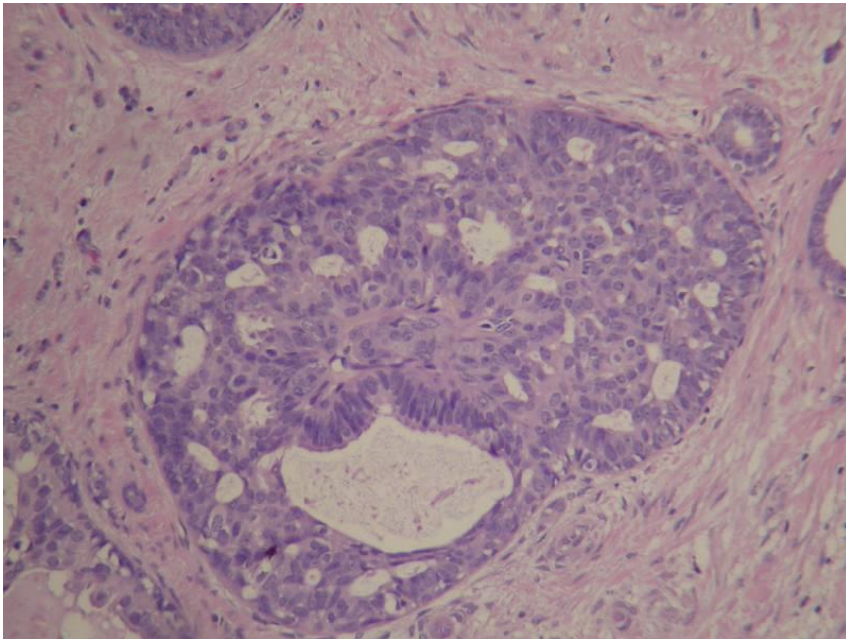
Myoepithelial markers

- smooth muscle myosin (SMMS1),
- Ck14
- p63
- CK5
- CK 5/6
- Basal membrane collagen IV

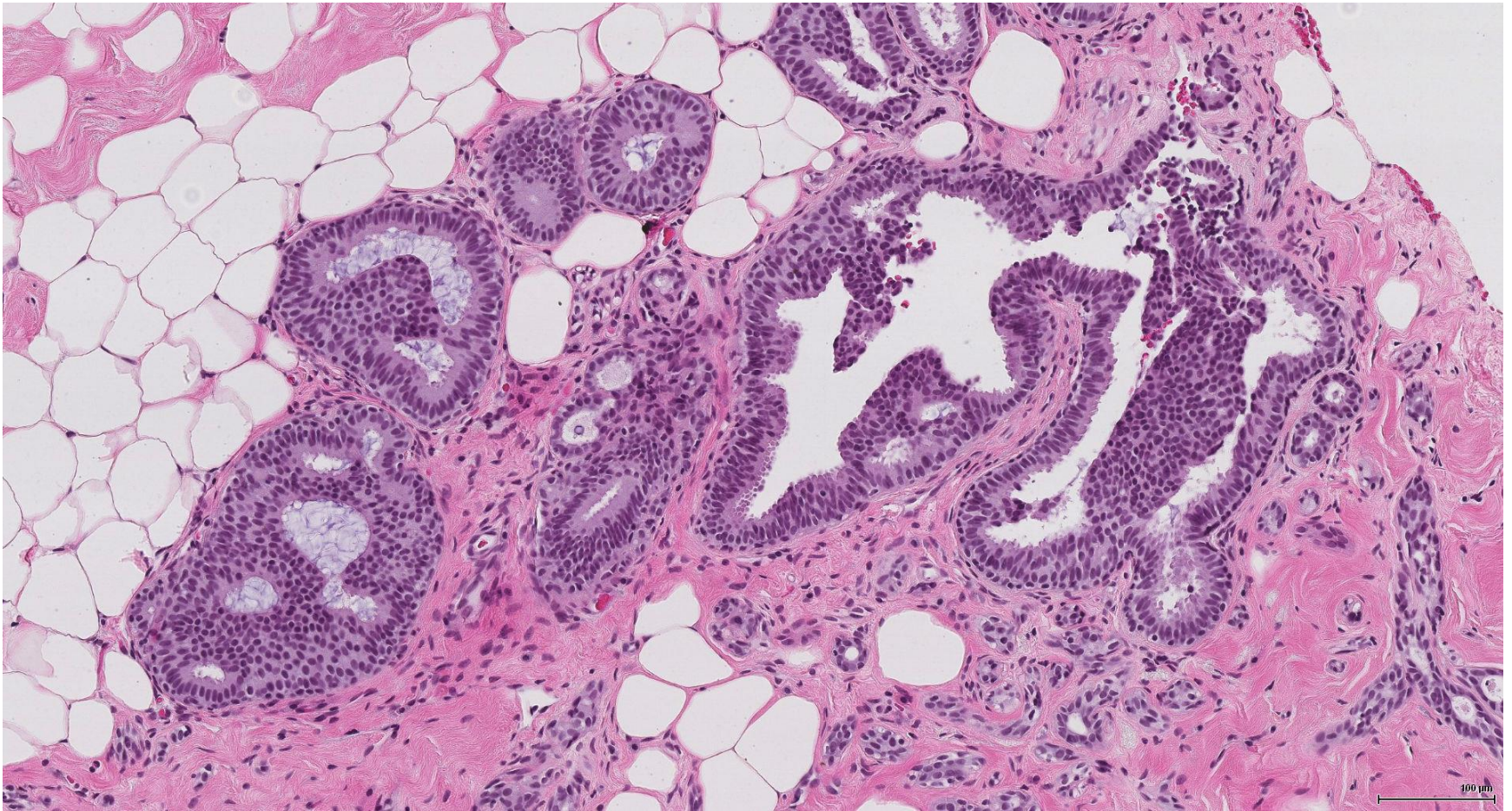
Immunohistochemical biomarkers for diagnosis

Benign ductal hyperplasia (UDH)

CK14 and or CK5/6



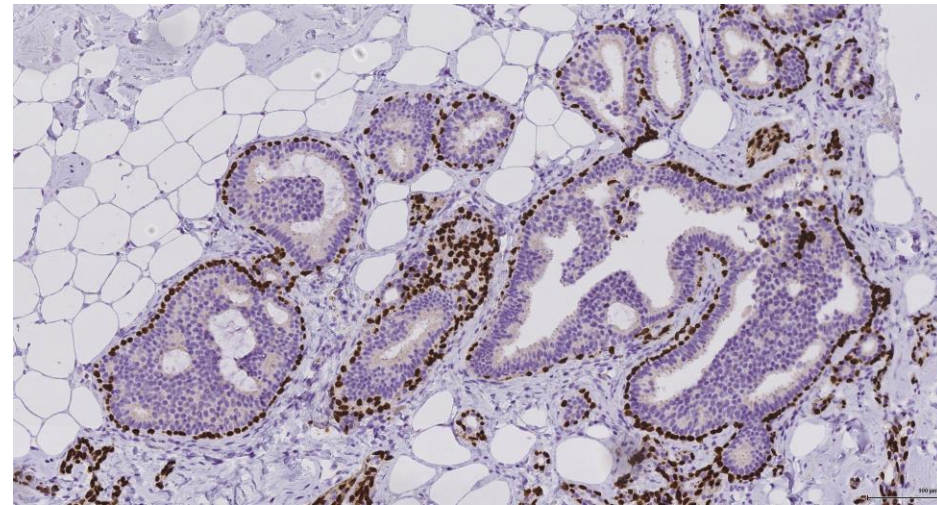
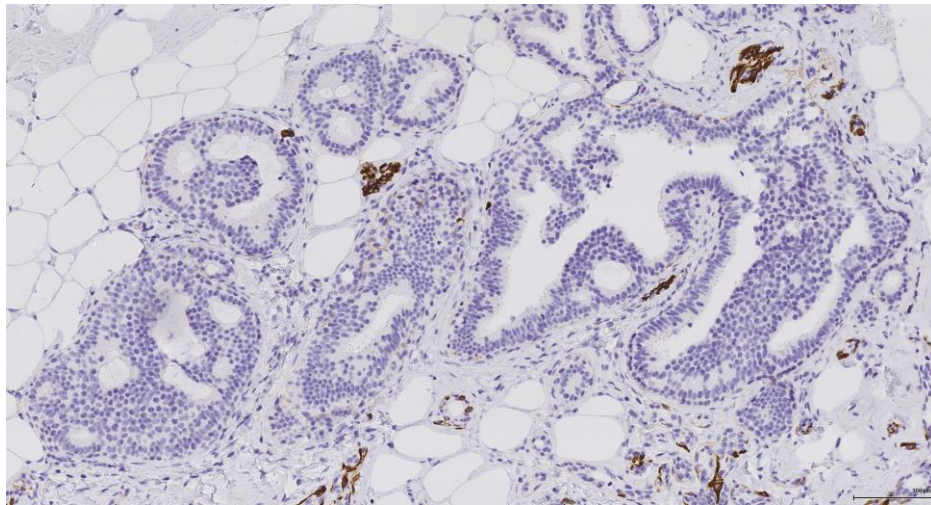
Atypical ductal Hyperplasia



Atypical Ductal Hyperplasia

CK14

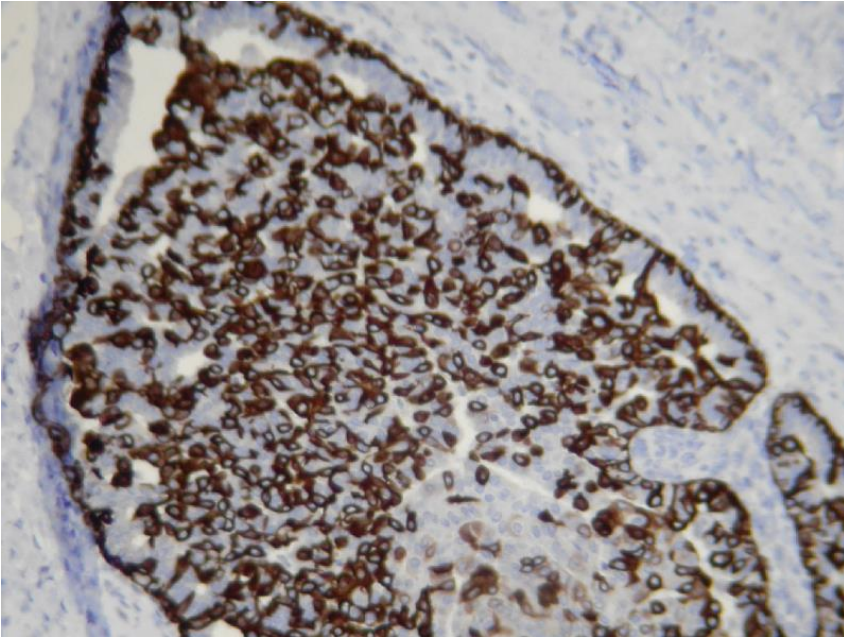
P63



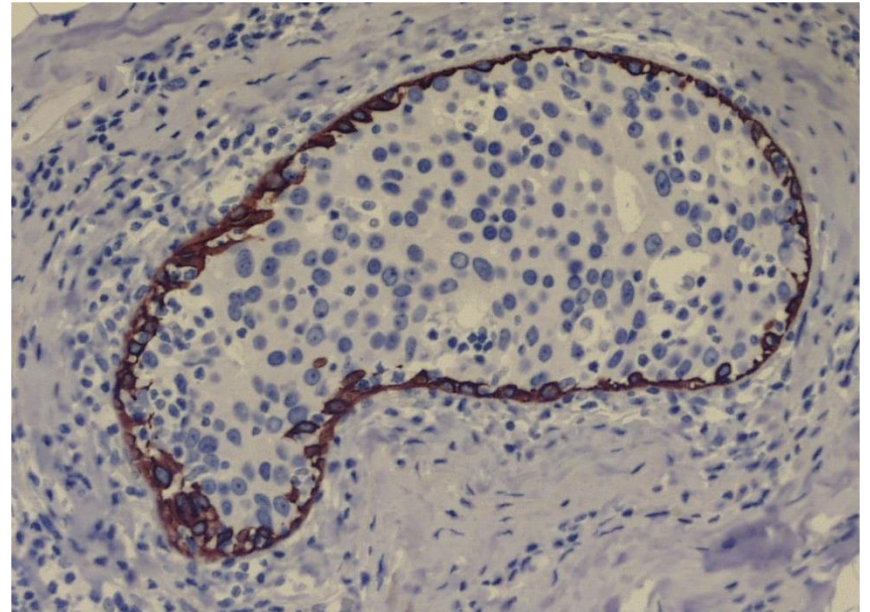
Always apply more than one immunohistochemical marker for myoepithelial cells

Ductal Carcinoma In Situ

CK14 Ductal Hyperplasia (UDH)

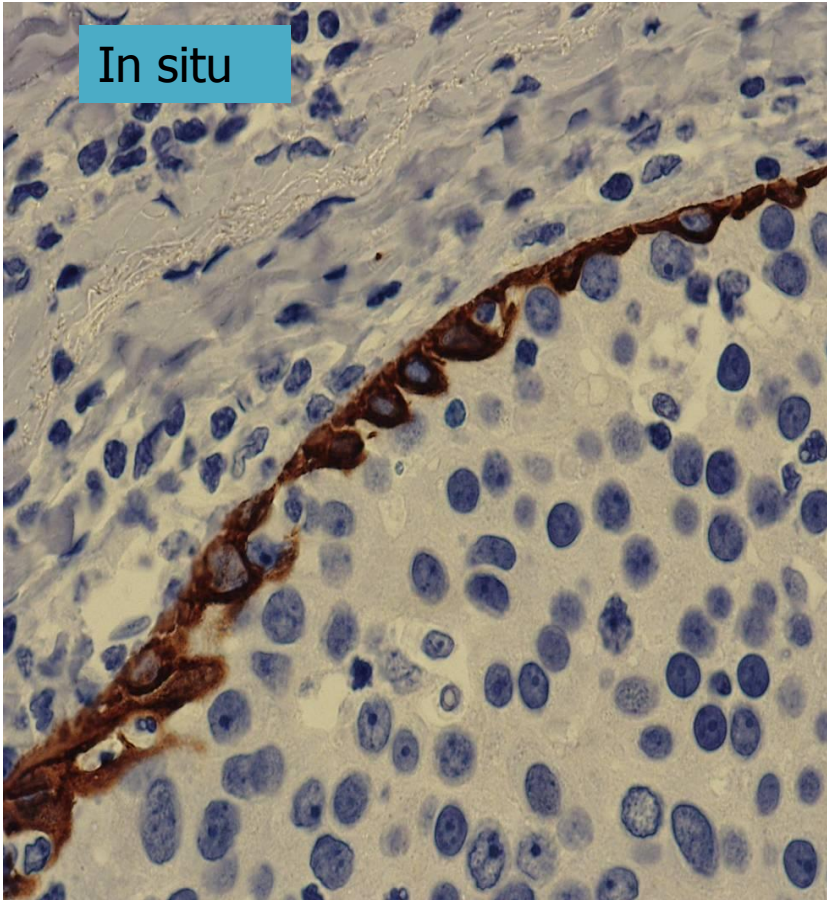


CK14 Ductal Carcinoma In Situ

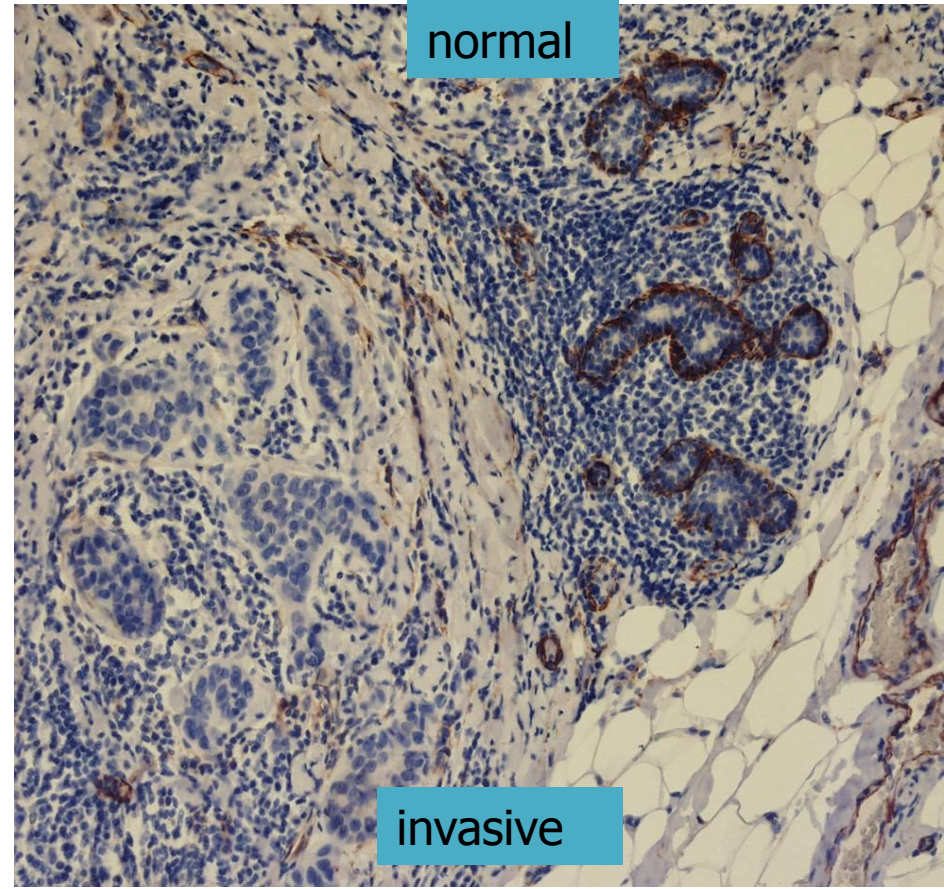


Invasive Carcinoma i.e. SMMS-1

present



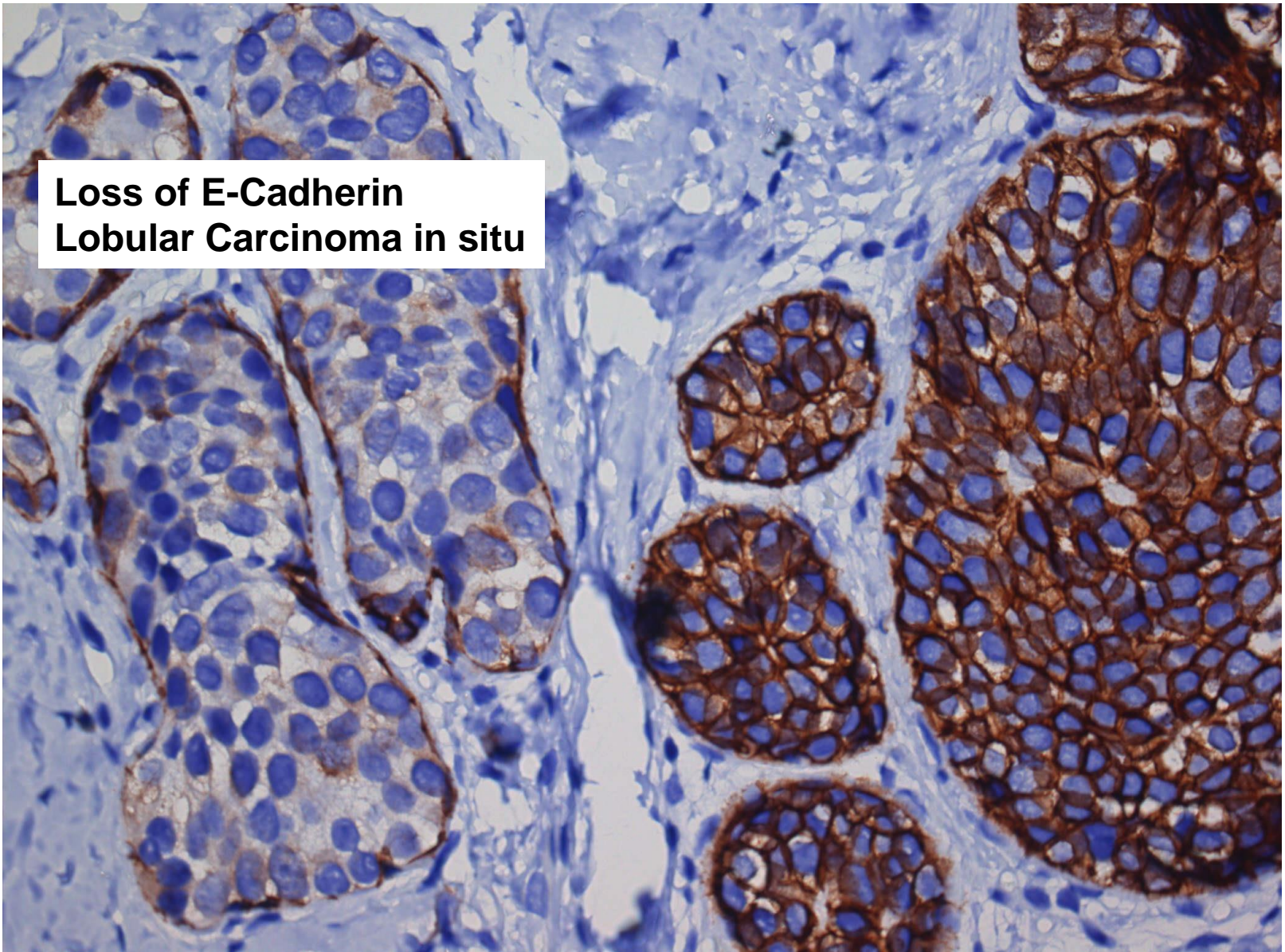
Not present



Detecting "presence"

Detecting "absence"

**Loss of E-Cadherin
Lobular Carcinoma in situ**

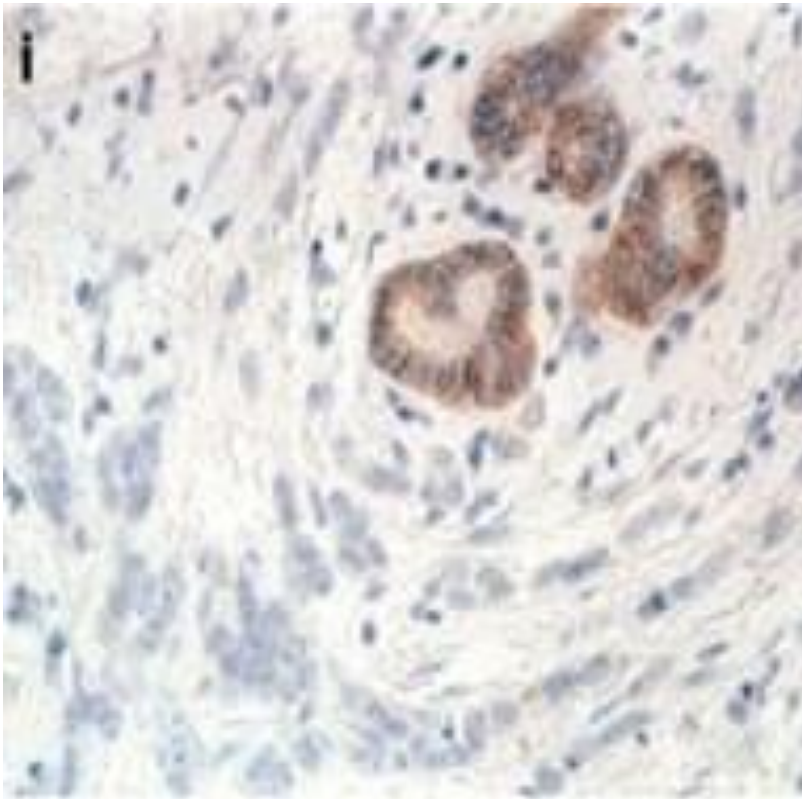


E-cadherin: Cell Adhesion Molecule

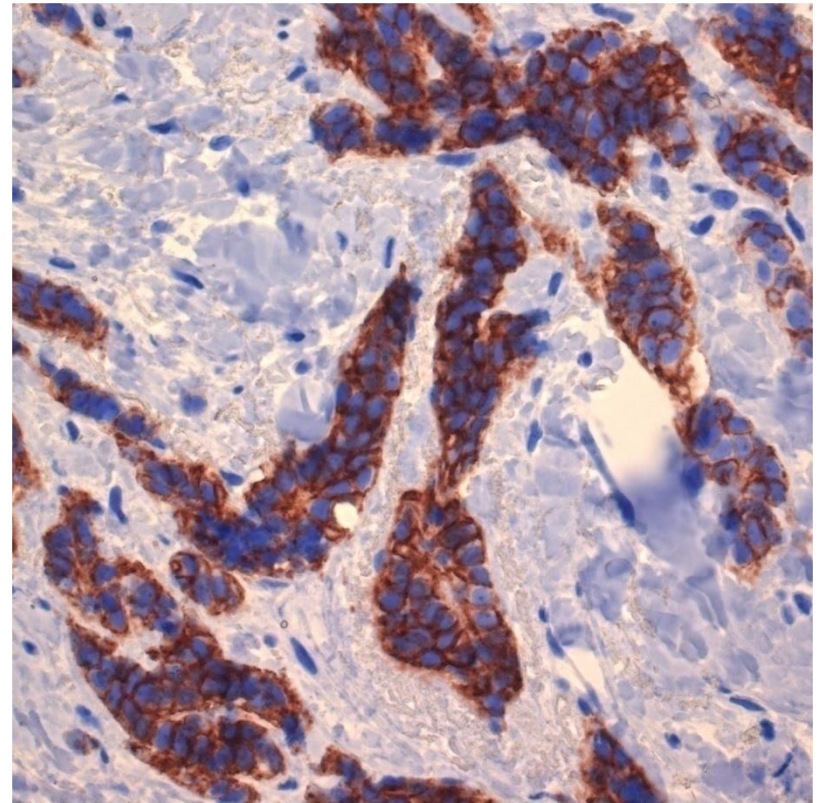
E-Cadherin

Cell adhesion molecule

**Loss of E-Cadherin in 90% of
Invasive lobular Carcinoma**

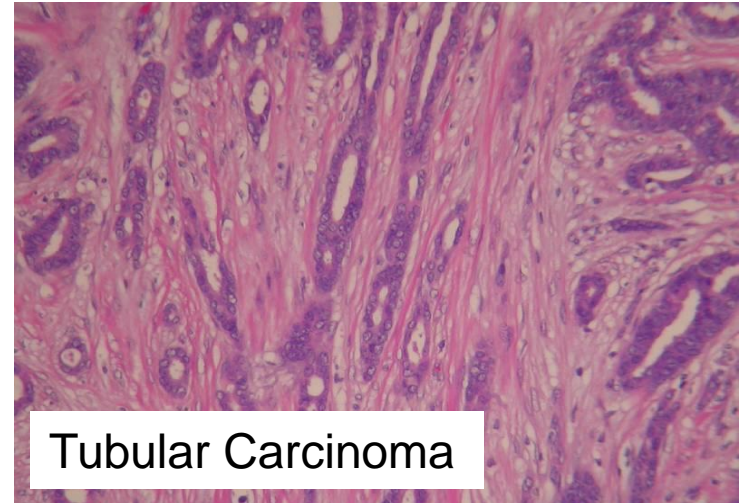


**E-Cadherin positive
Invasive Ductal Carcinoma**

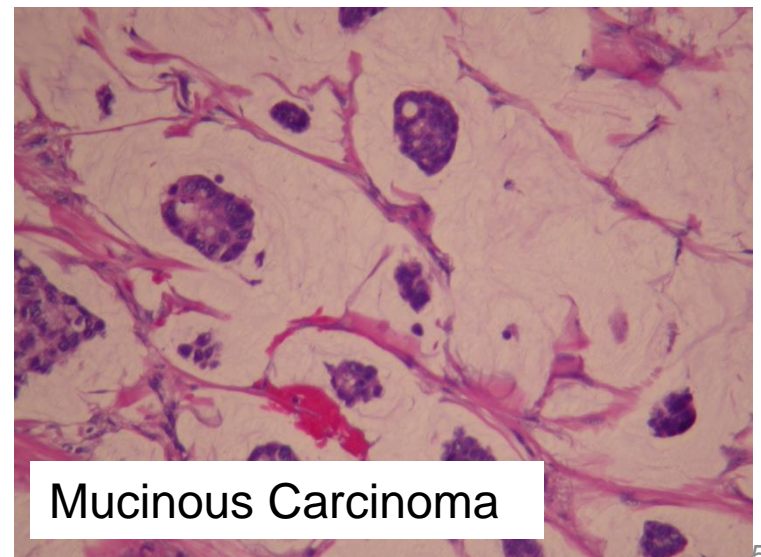


Invasive Breast Cancer Histological Subtypes

- Ductal : up to 80%
- Lobular: 5 - 14%
- Tubular: 2 - 8%
- Mucinous: 2 - 4 %
- Apocrine: 1 – 4%
- Papillary 1 – 2%
- Other



Tubular Carcinoma

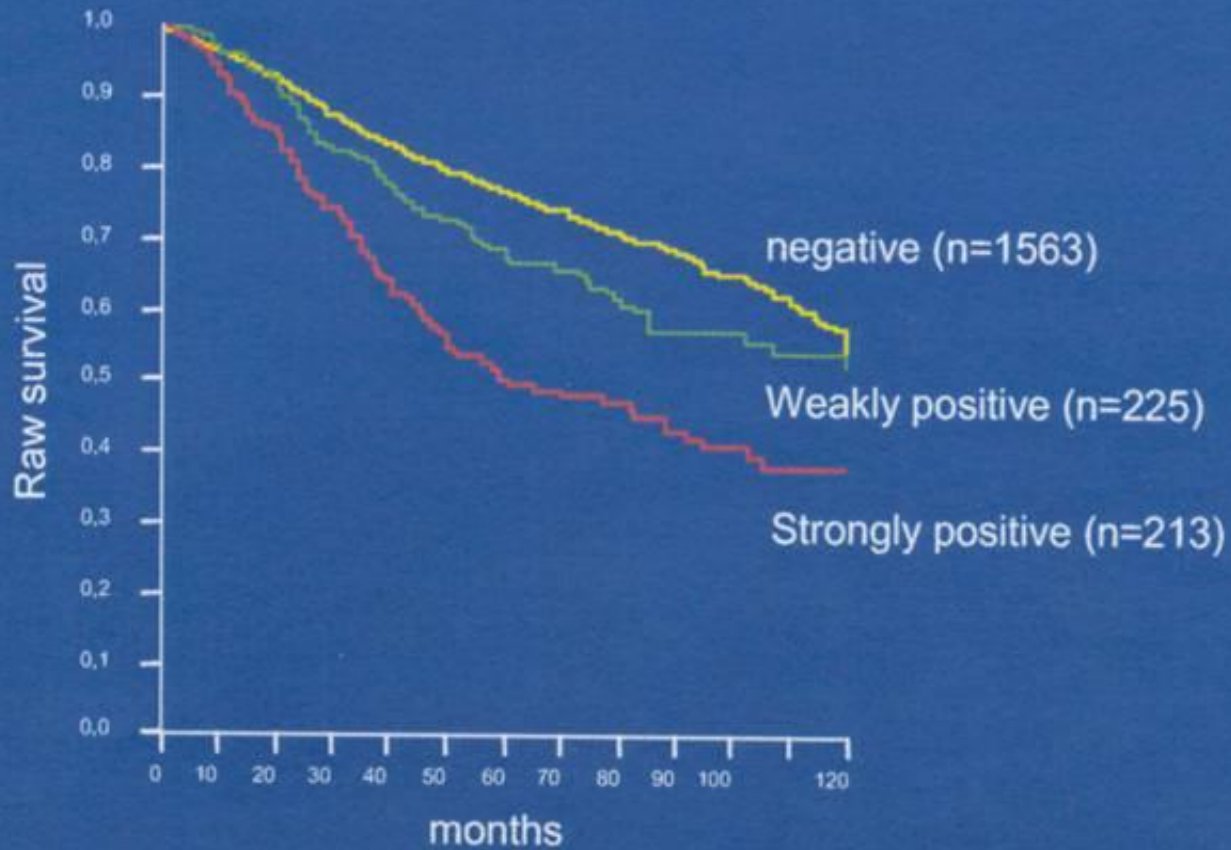


Mucinous Carcinoma

Agenda

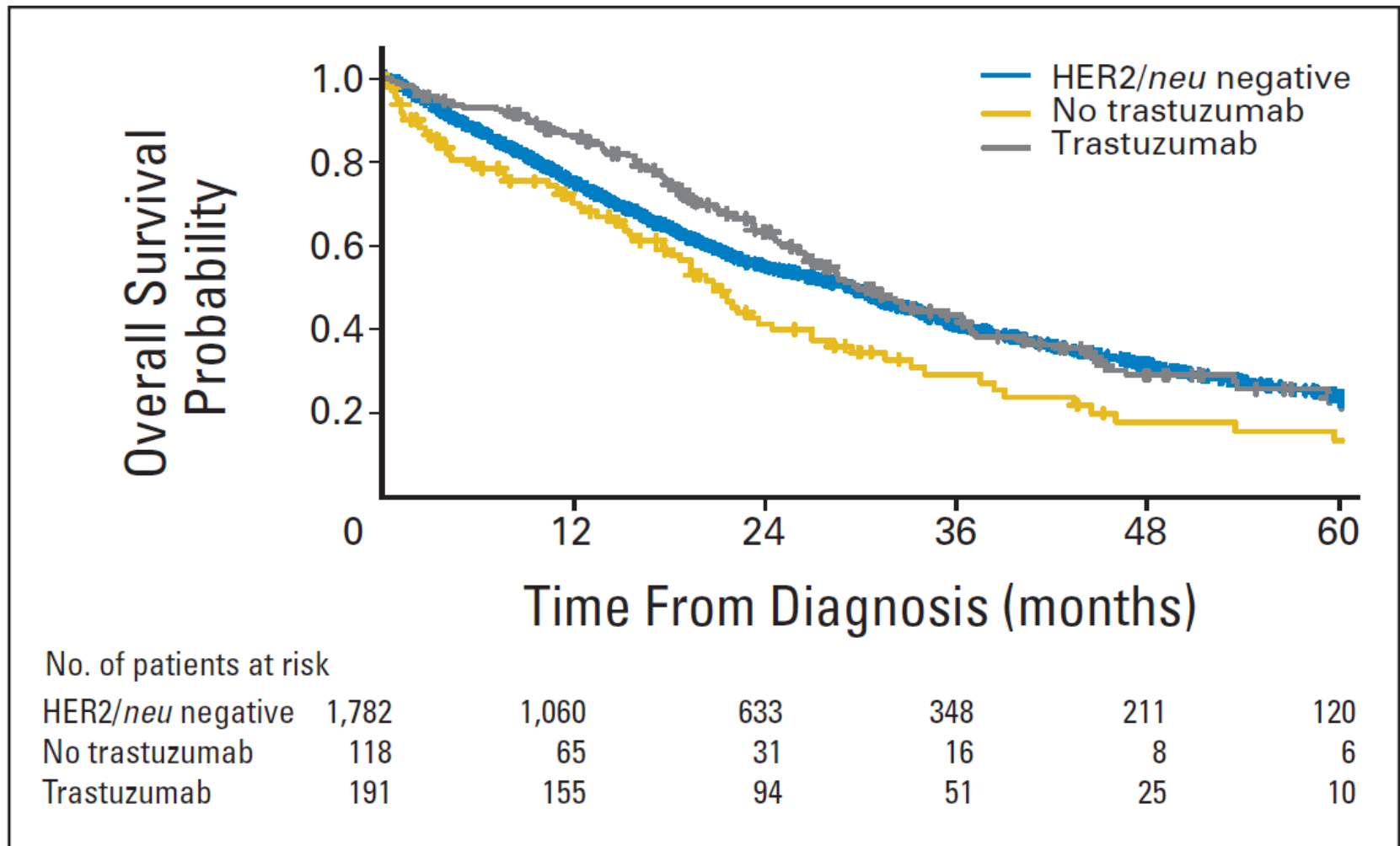
- Immunohistochemical biomarkers for
 - Diagnostics
 - Benign Hyperplasia versus Ductal Carcinoma In Situ
 - Ductal Carcinoma in Situ versus Lobular Carcinoma in Situ
 - Carcinoma In Situ versus Invasive Carcinoma
 - Invasive carcinoma
 - Ductal versus Lobular
 - **Prediction/Prognosis**
 - **Estrogen Receptor**
 - **Progesteron Receptor**
 - **HER2**
 - **Ki67**
 - Intrinsic Subtype Classification

HER2 and Breast Cancer Progression

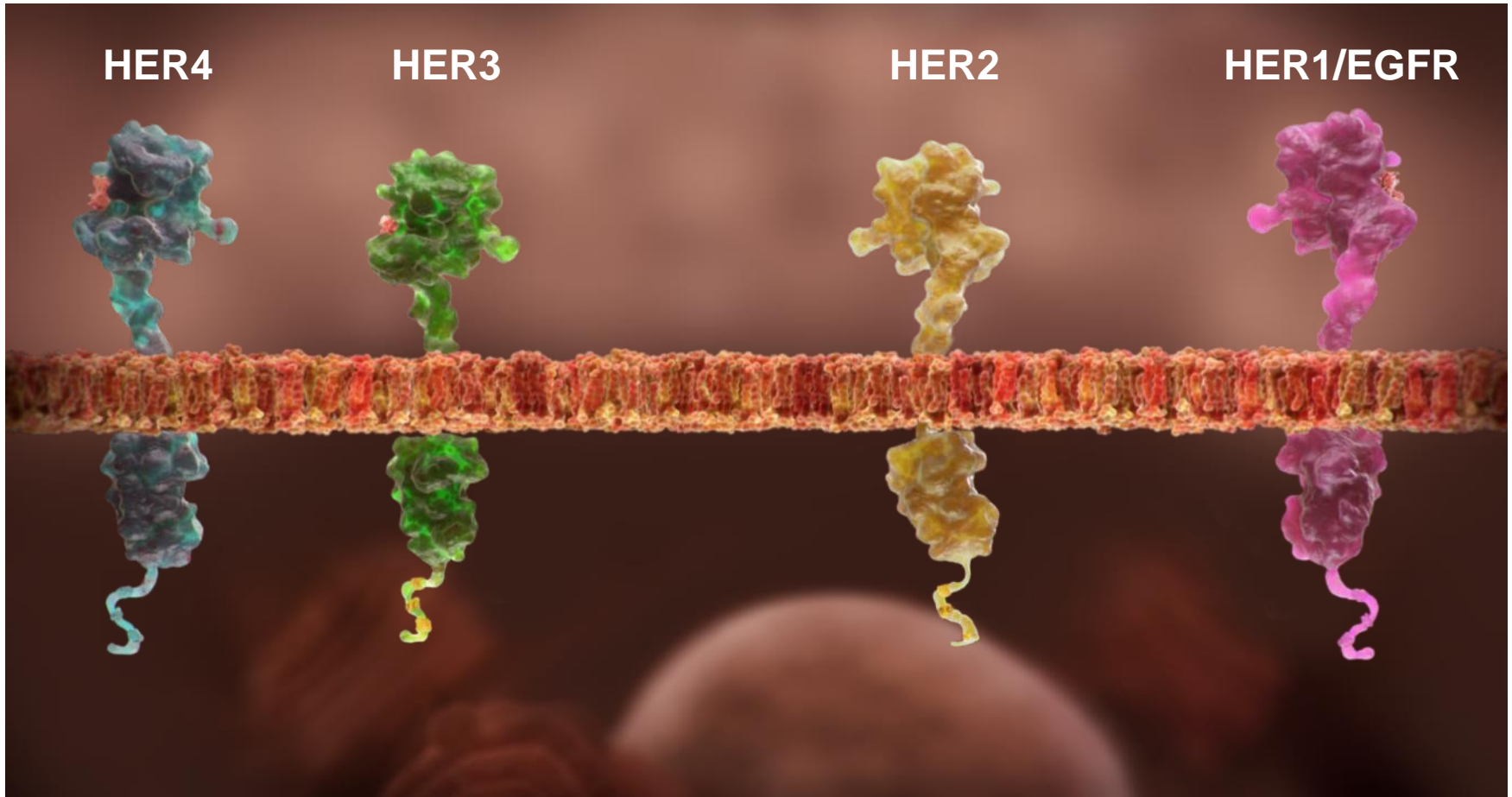


Science, Vol 235, 1987

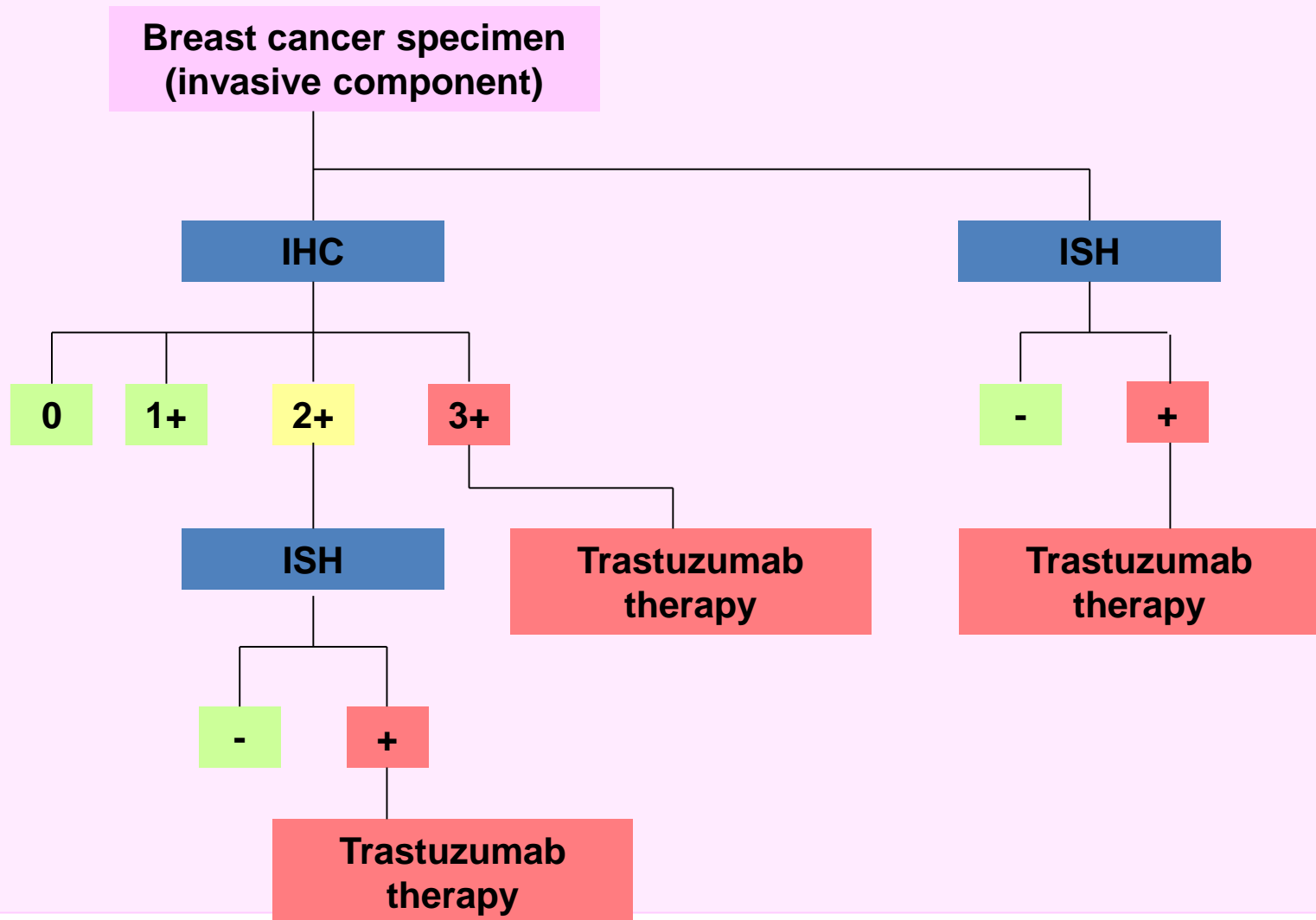
HER2 targeting has changed the natural history of HER2-positive advanced breast cancer



There are four receptors in the HER family



HER2 Algorithm



Two different assays

- IHC is an assay at the single-cell level
 - It will detect even an individual positive cell
- ISH is a population-based assay (mean number of gene copies/cell evaluated by scoring 20-60 cells.)
 - The final result depends on the number of gene copies of the amplified cells after dilution by non-amplified cells

Published Ahead of Print on October 7, 2013 as 10.1200/JCO.2013.50.9984
The latest version is at <http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2013.50.9984>

JOURNAL OF CLINICAL ONCOLOGY

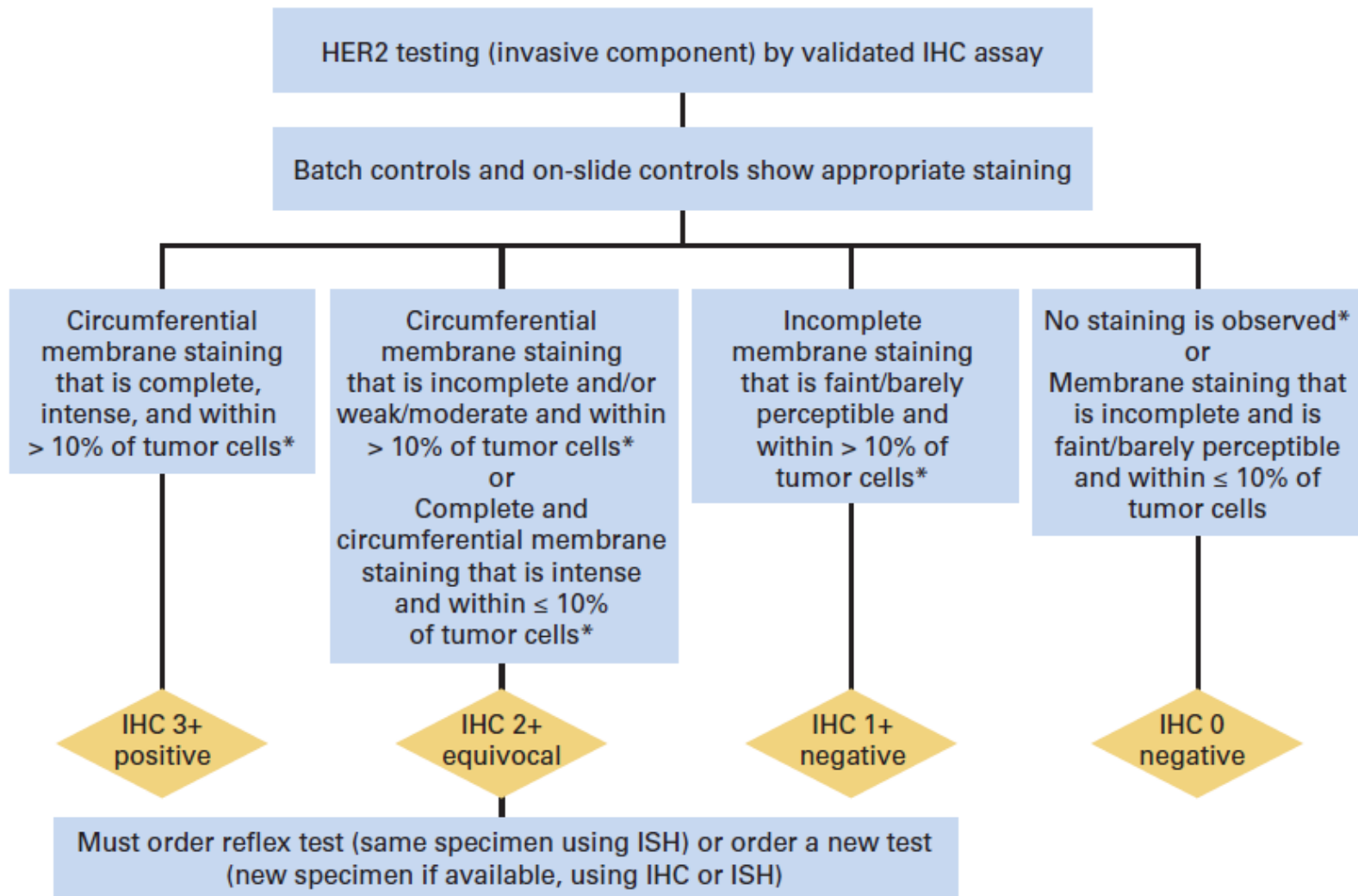
A S C O S P E C I A L A R T I C L E

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

Antonio C. Wolff, M. Elizabeth H. Hammond,* David G. Hicks,* Mitch Dowsett,* Lisa M. McShane,* Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes**

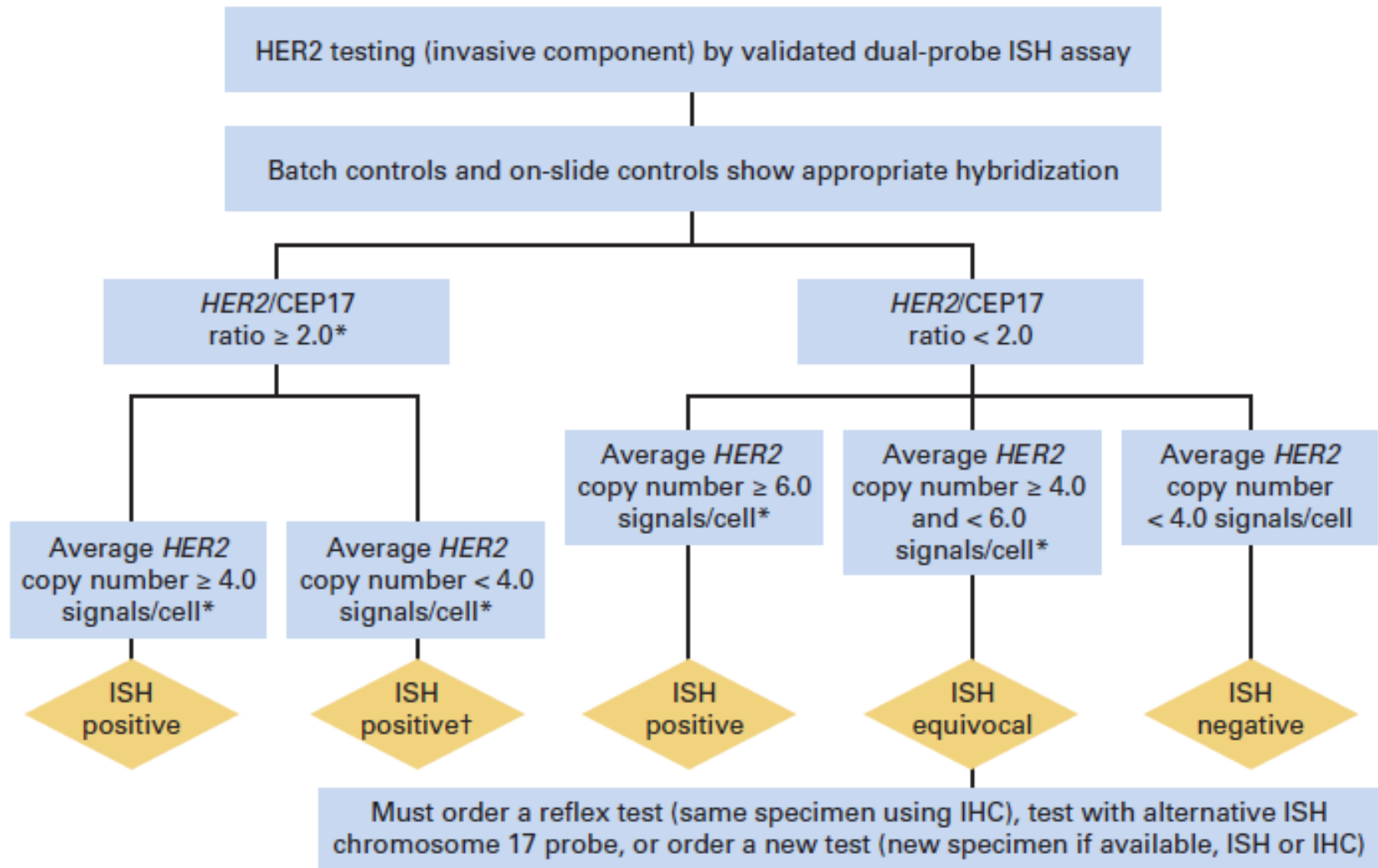
HER2 Testing IHC

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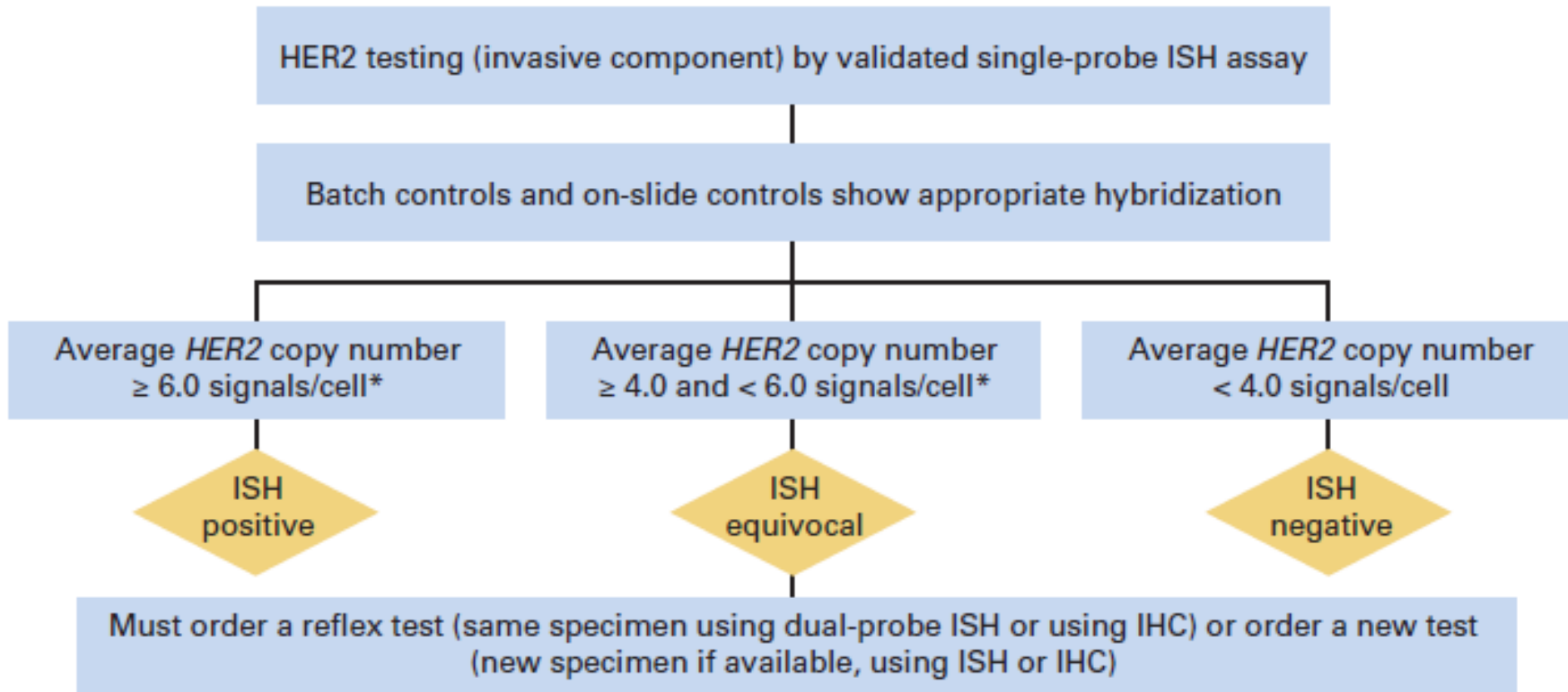
HER2 Testing by Dual Probe

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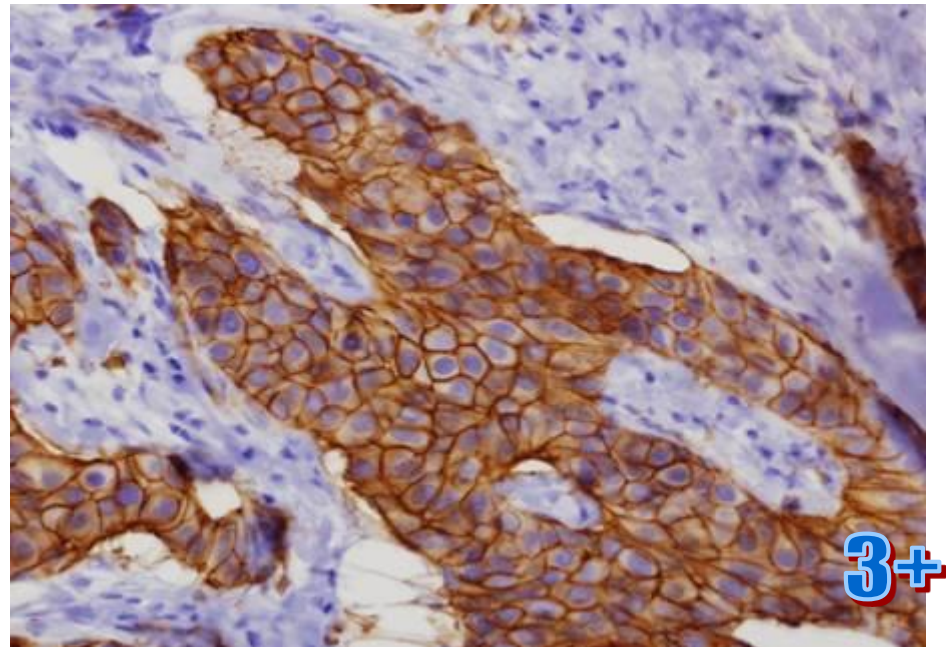
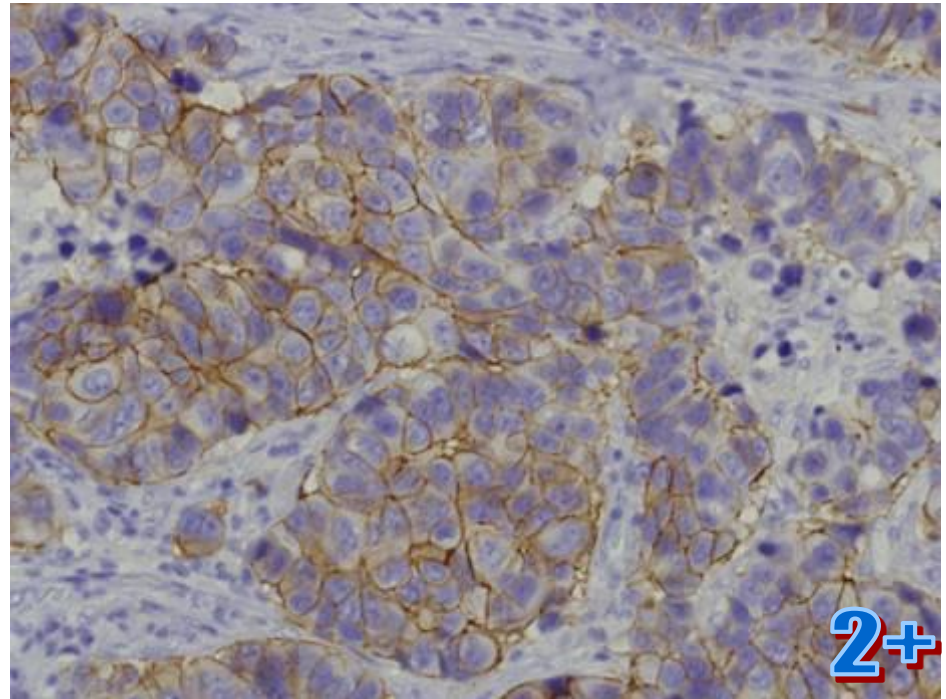
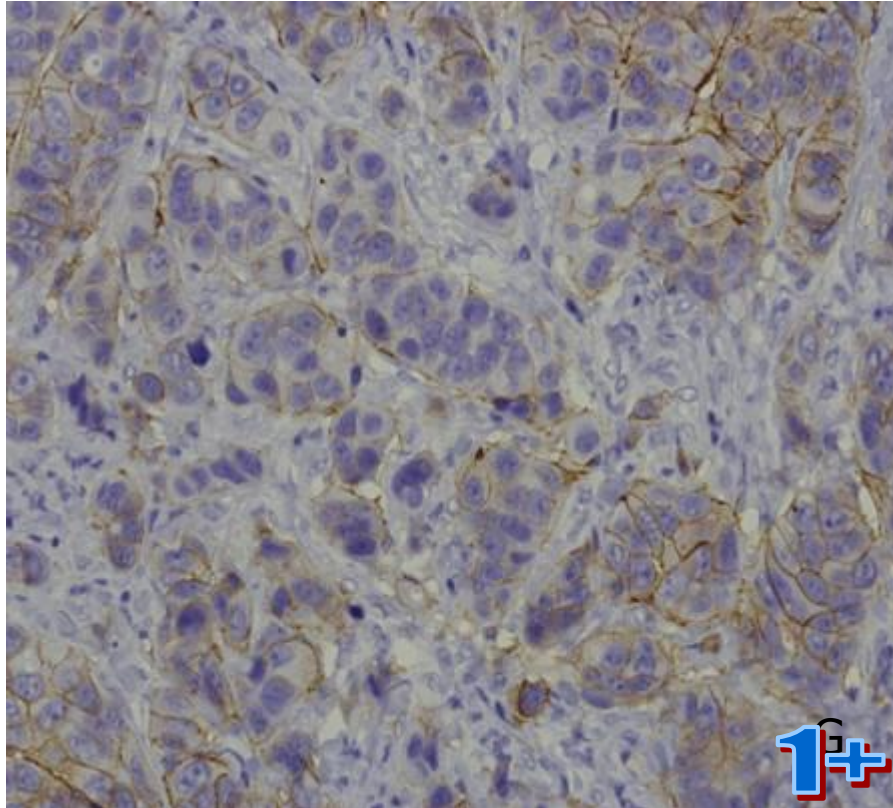


HER2 Testing Single Probe ISH

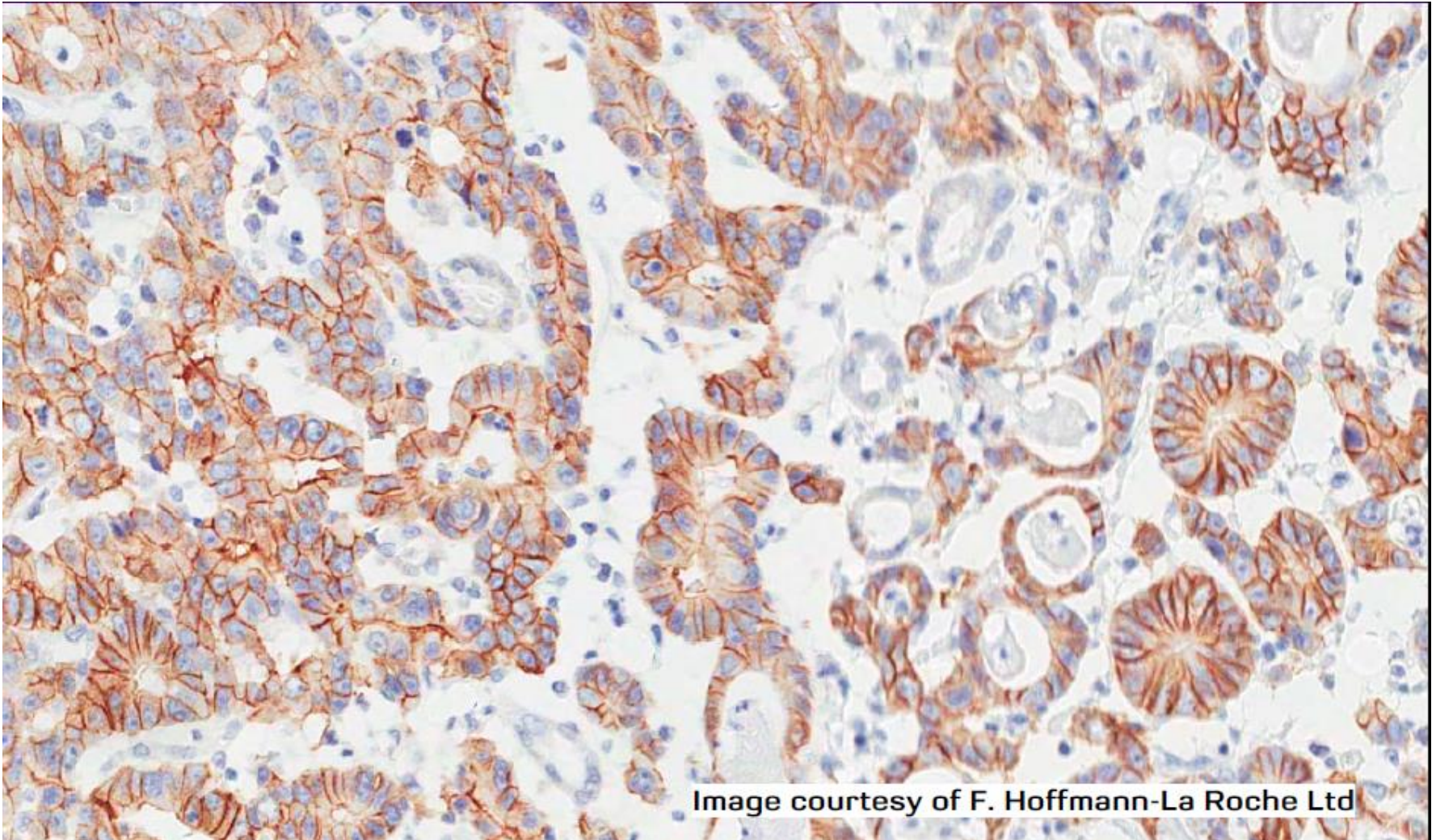
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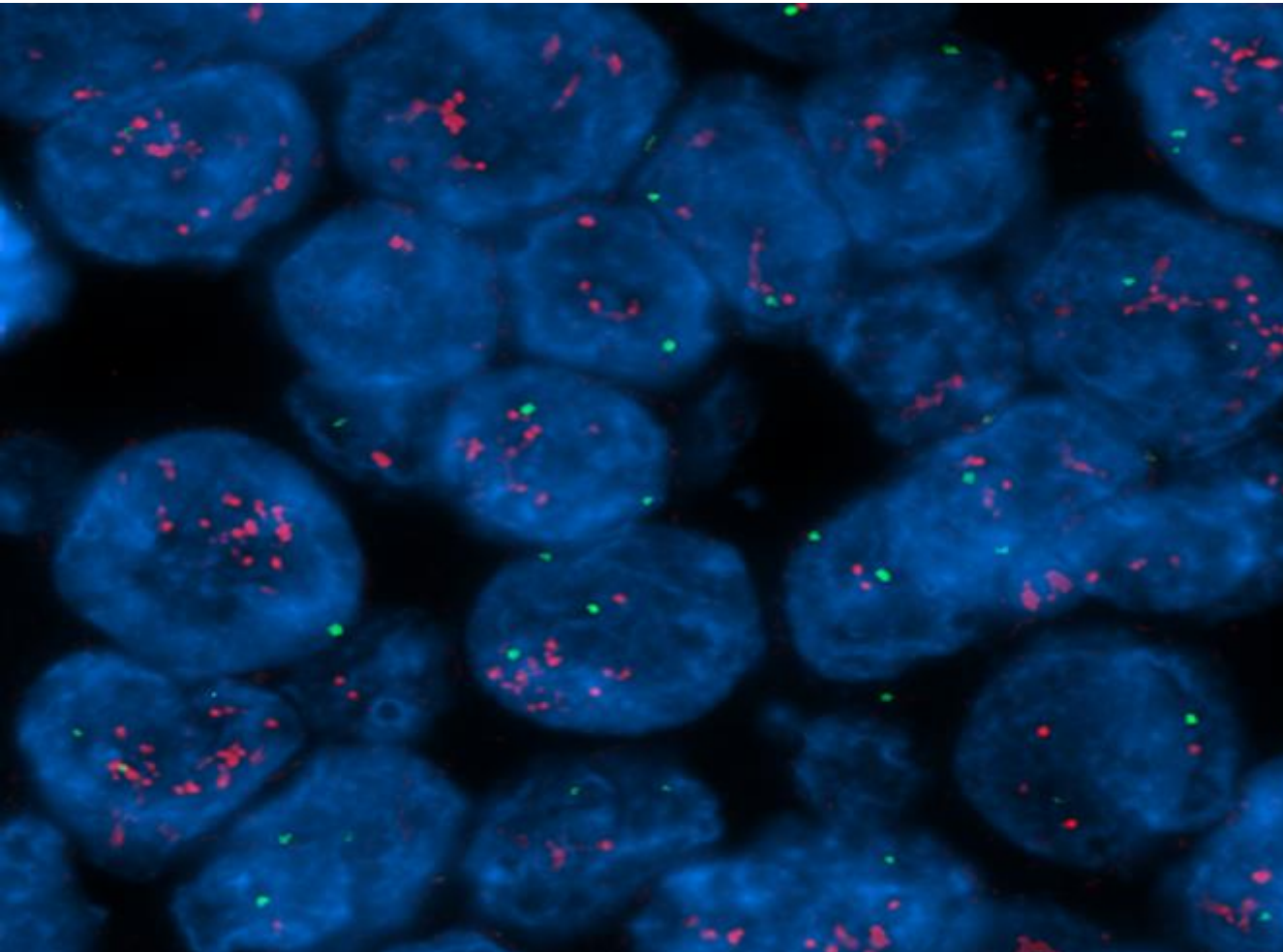
HER2 IHC



HER2 2+ basolateral staining



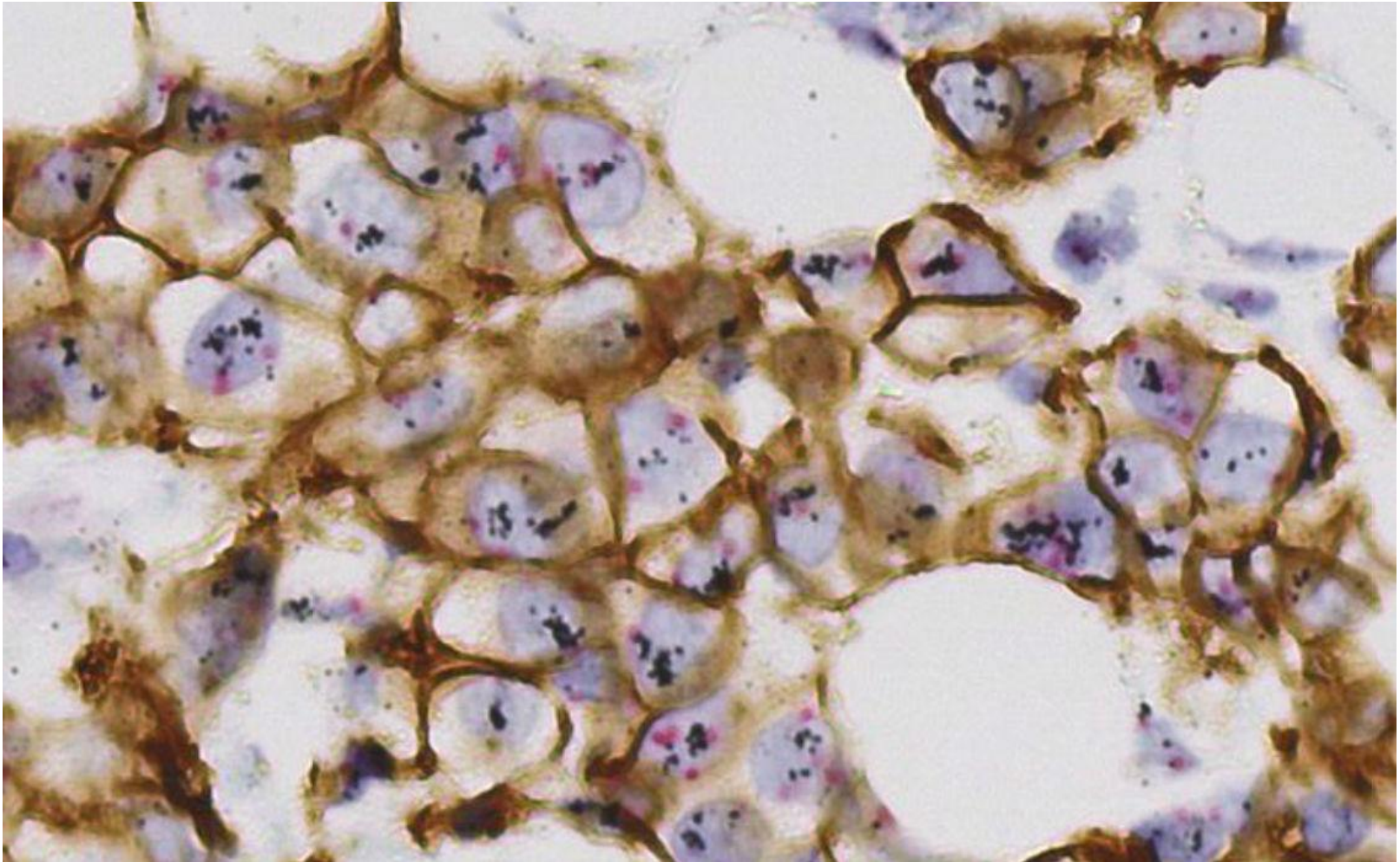
HER2 FISH



Green: :centromere
chromosome 17

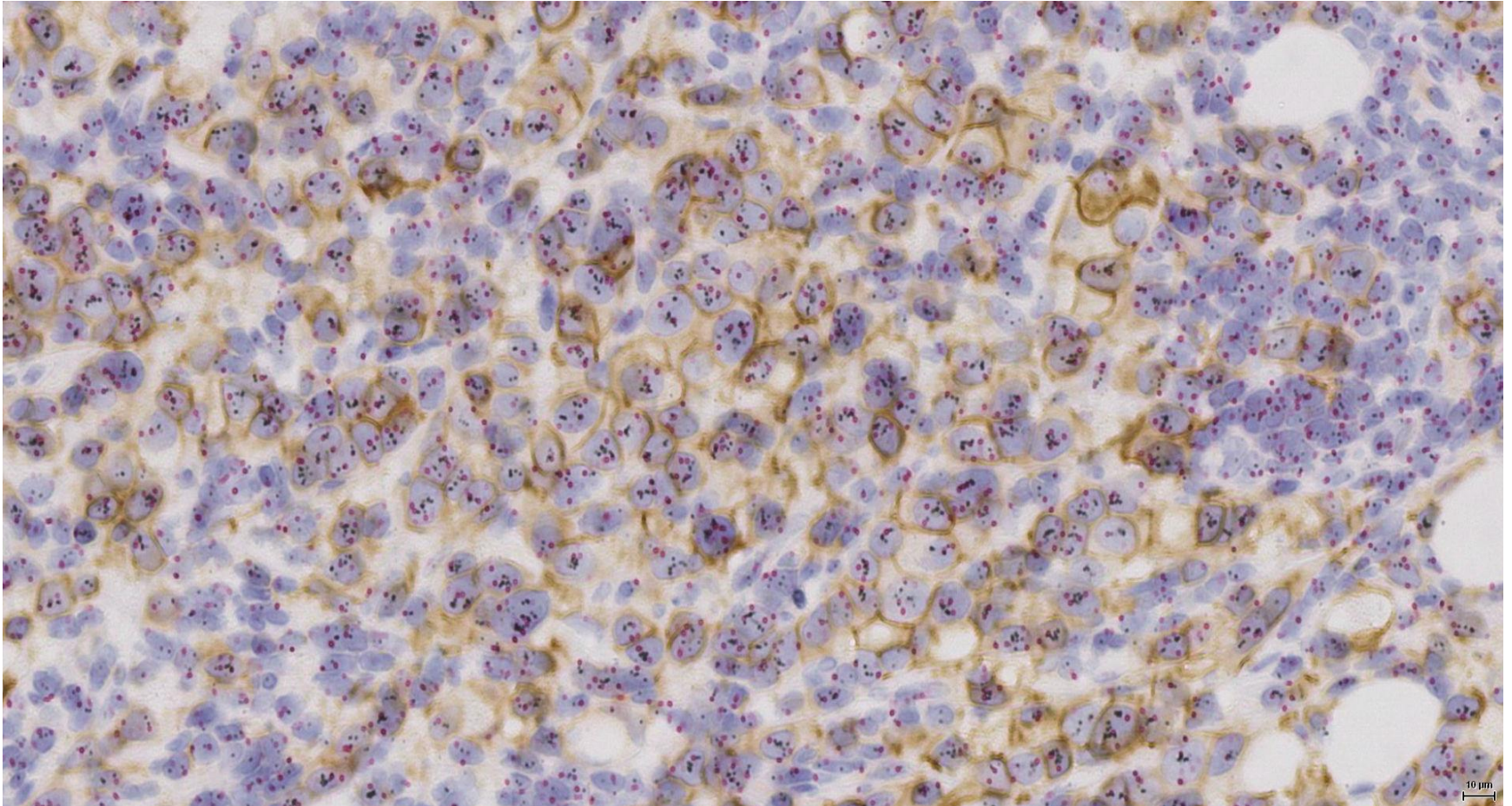
Red : HER2 gene

HER2 Gene/Protein Assay



HER2 amplified and HER2 IHC 3+

Intratumour heterogeneity HER2 Breast

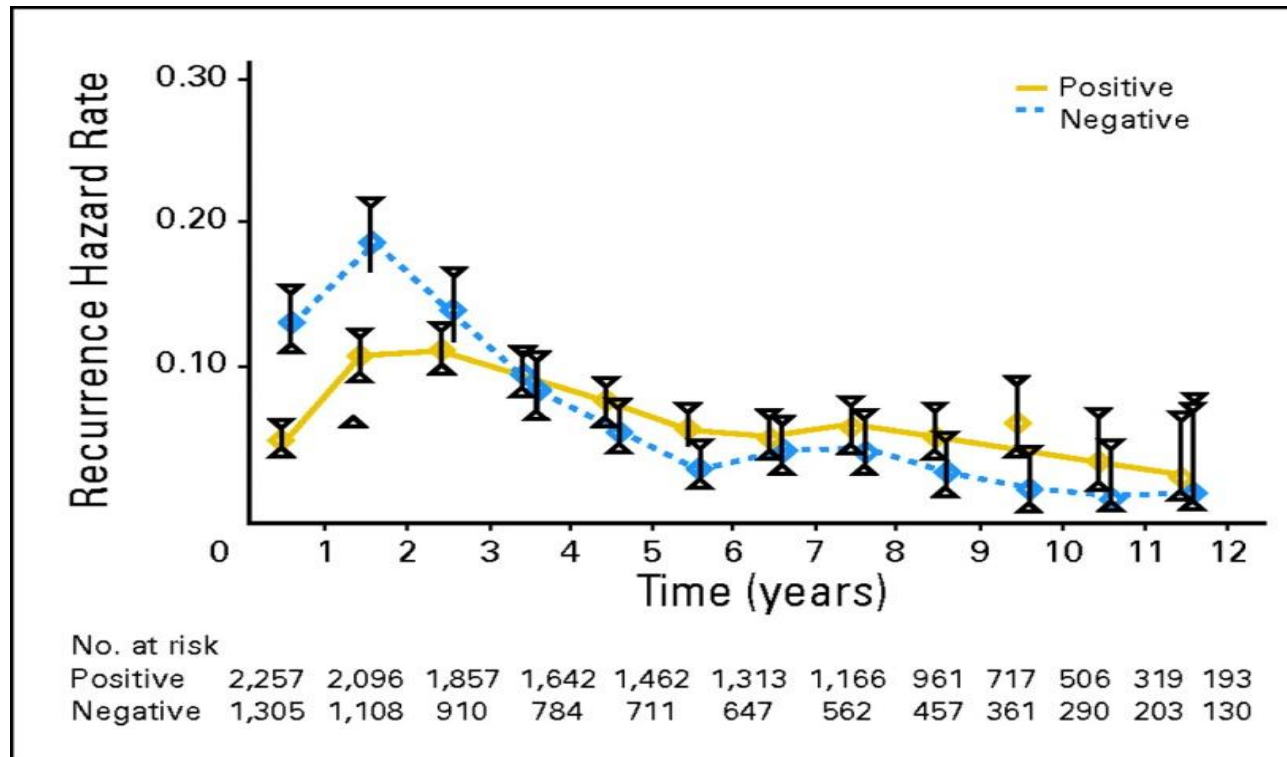


HER2 Gene/Protein Assay

The oestrogen receptor as a prognostic marker

Shift from prognostic to predictive!!

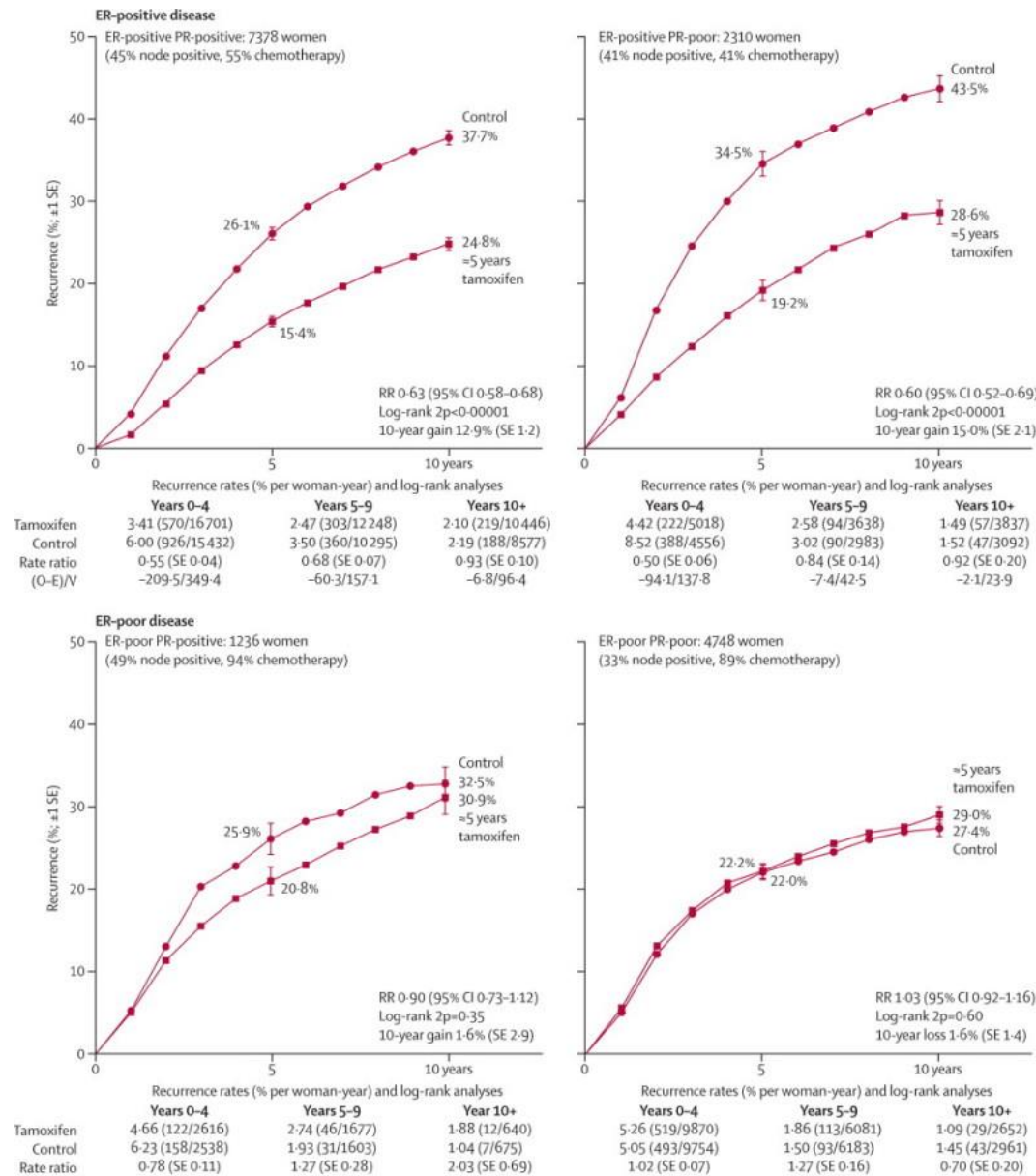
Risk of recurrence pr. year
N = 3,562 patients



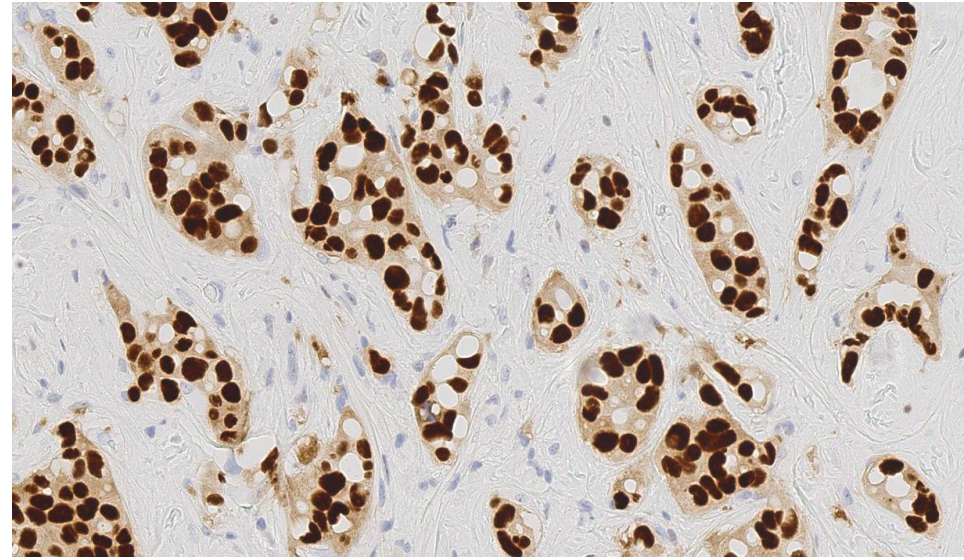
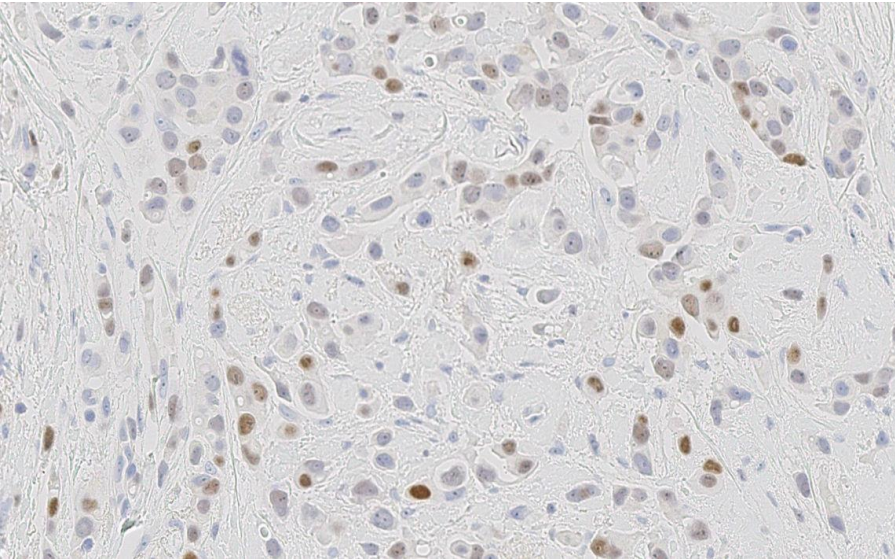
Lin, N. U. et al. J Clin Oncol; 26:798-805 2008

Relevance of measured ER and PR status on the effects of about 5 years of tamoxifen on the 10 year probability of recurrence (EBCTCG)

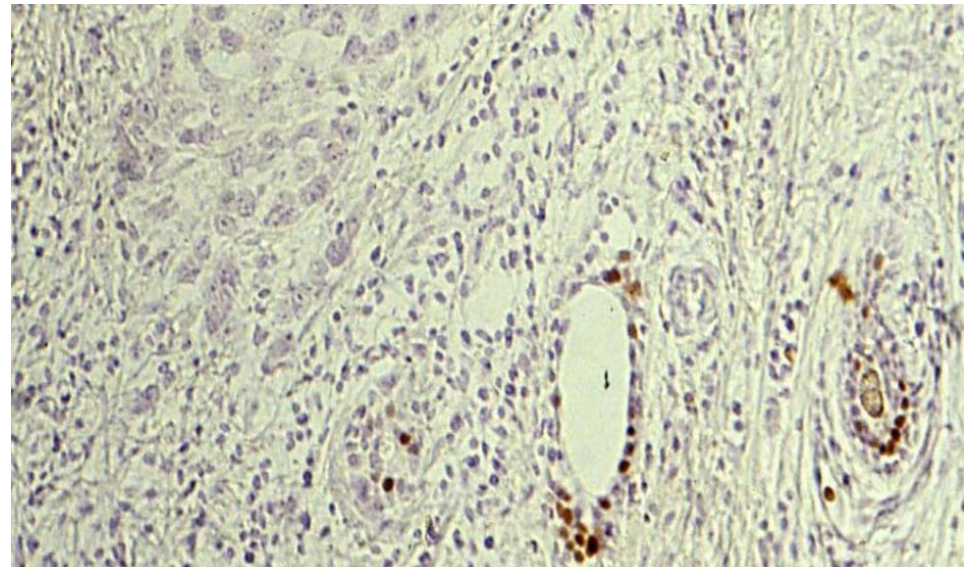
Lancet. 2011 August 27; 378(9793): 771–784.



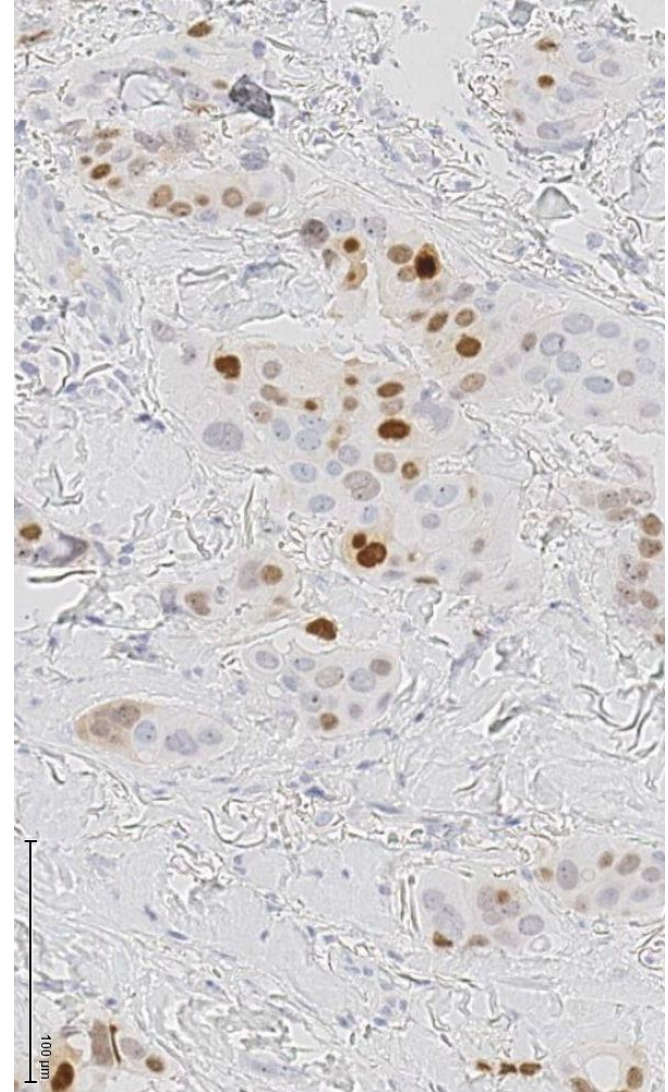
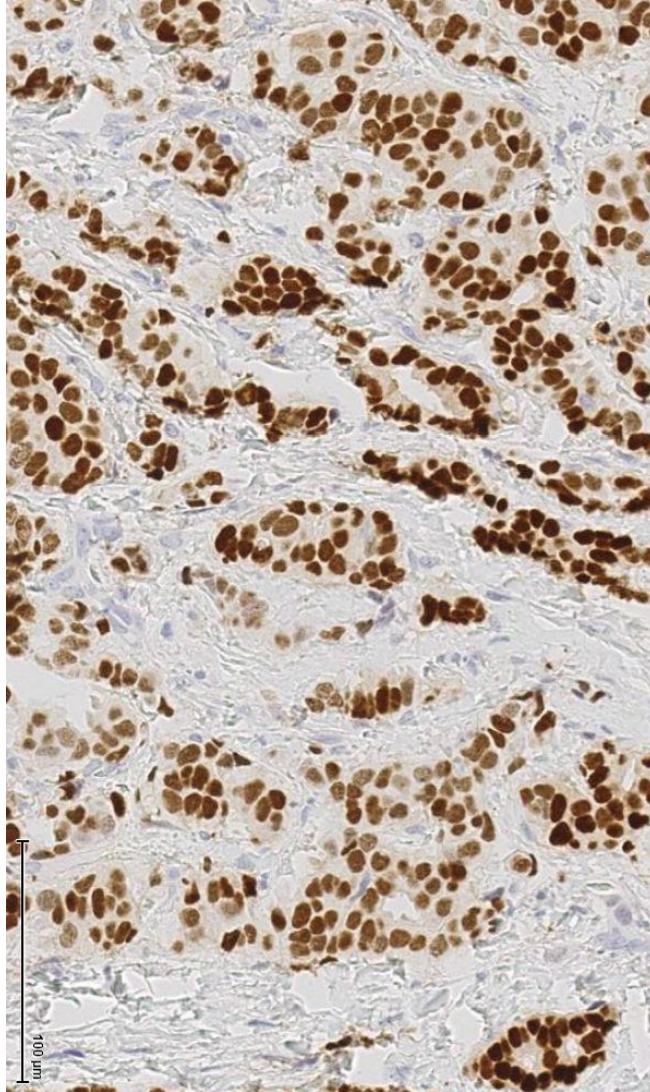
Interpretation of ER IHC



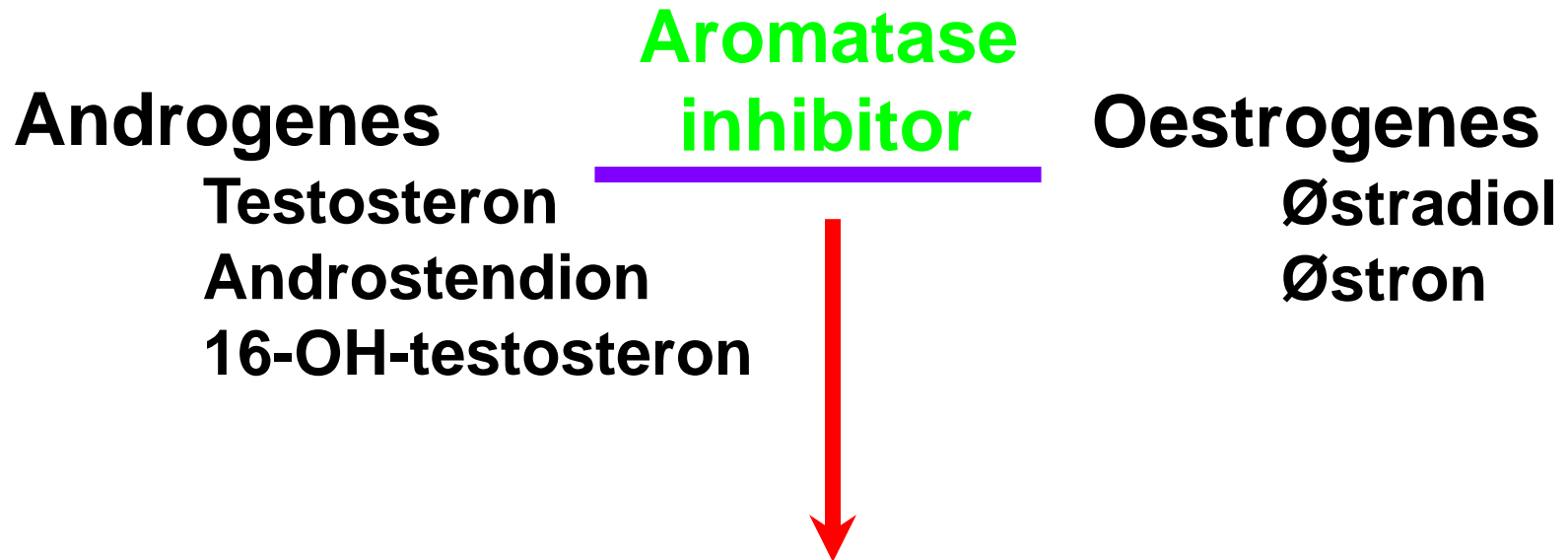
ER positive > 80% of breast carcinomas
Cut off $\geq 1\%$



Interpretation of PgR IHC

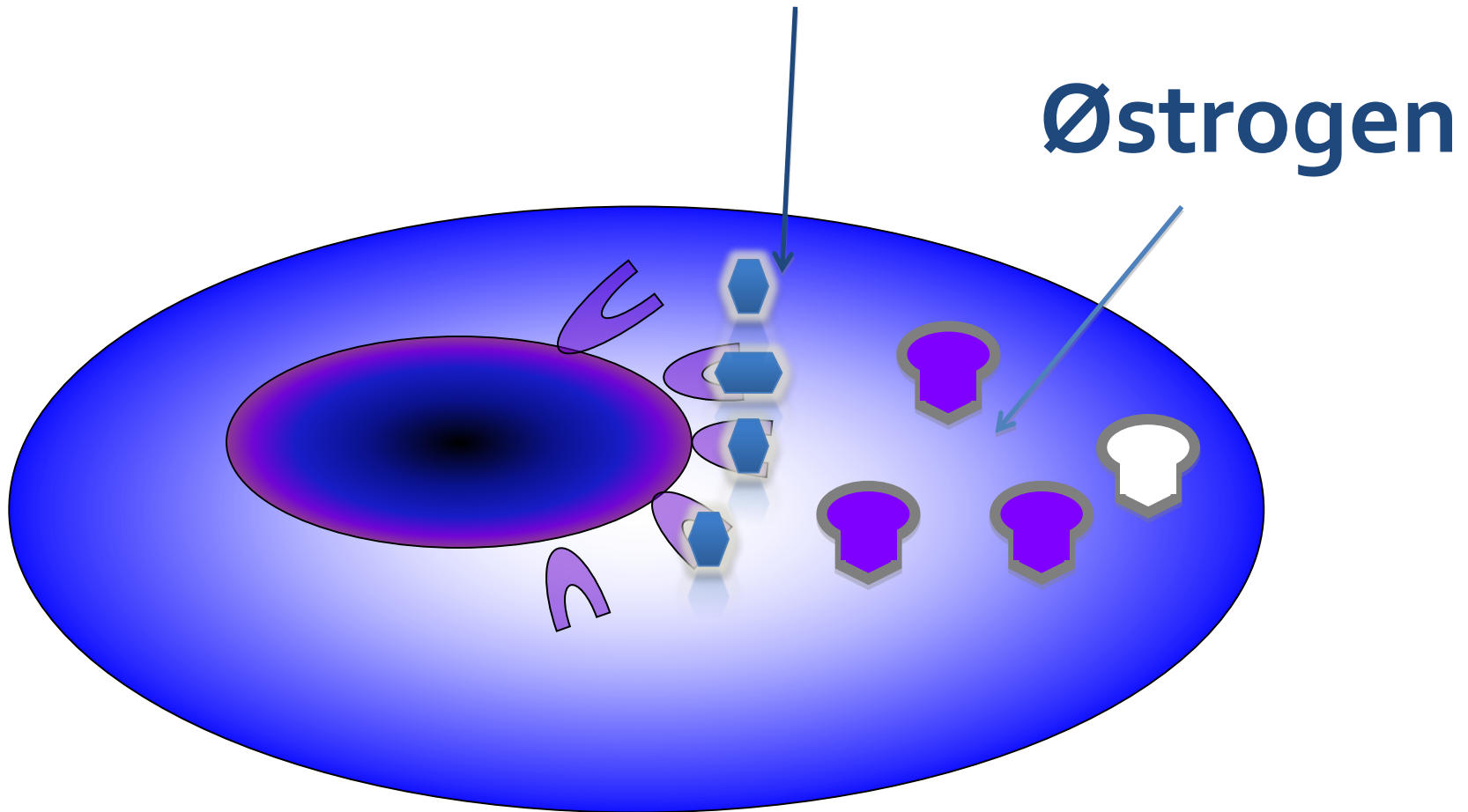


Aromatase-inhibitor



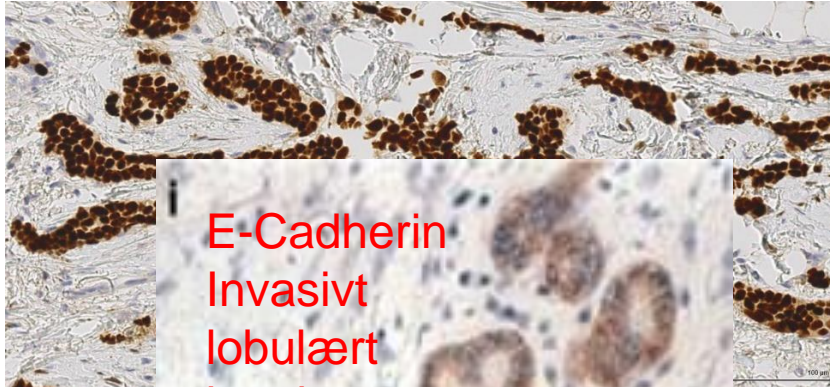
**Inhibit synthesis of oestrogens
in postmenopausal women**

Tamoxifen

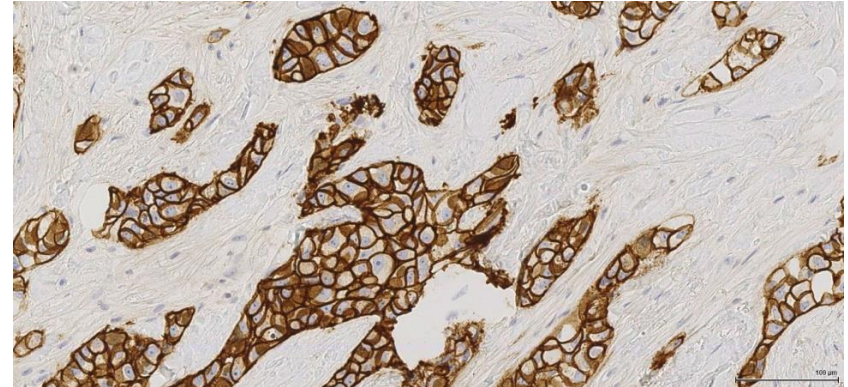


Receptor status - Denmark

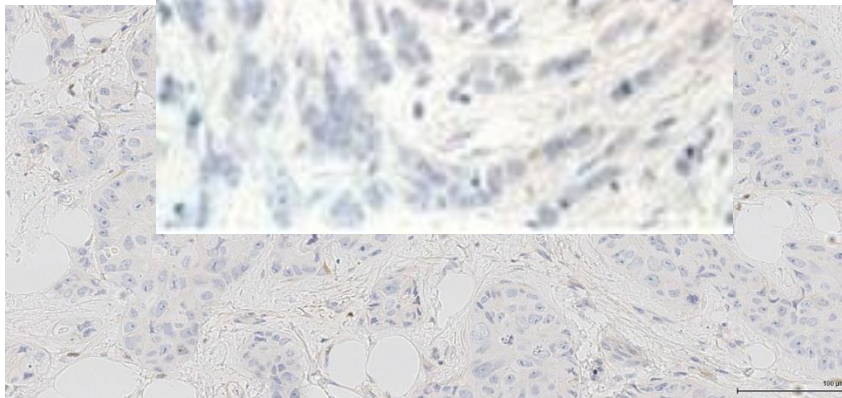
ER + : 86 %



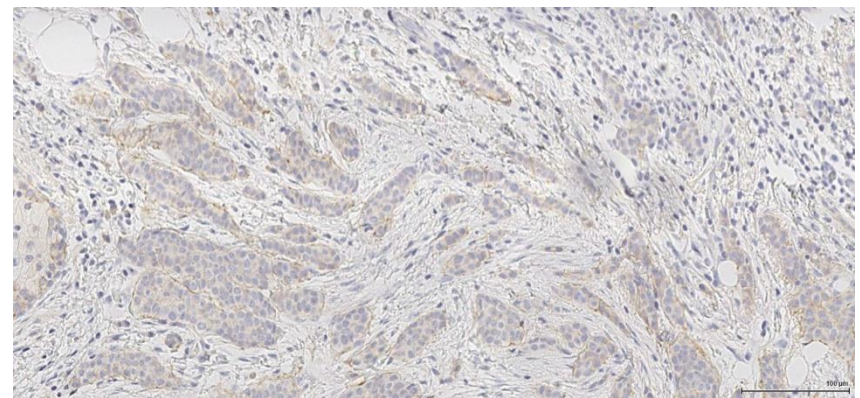
HER2 + : 14 %



ER -



HER2 -



ER and HER2 negative: 10%

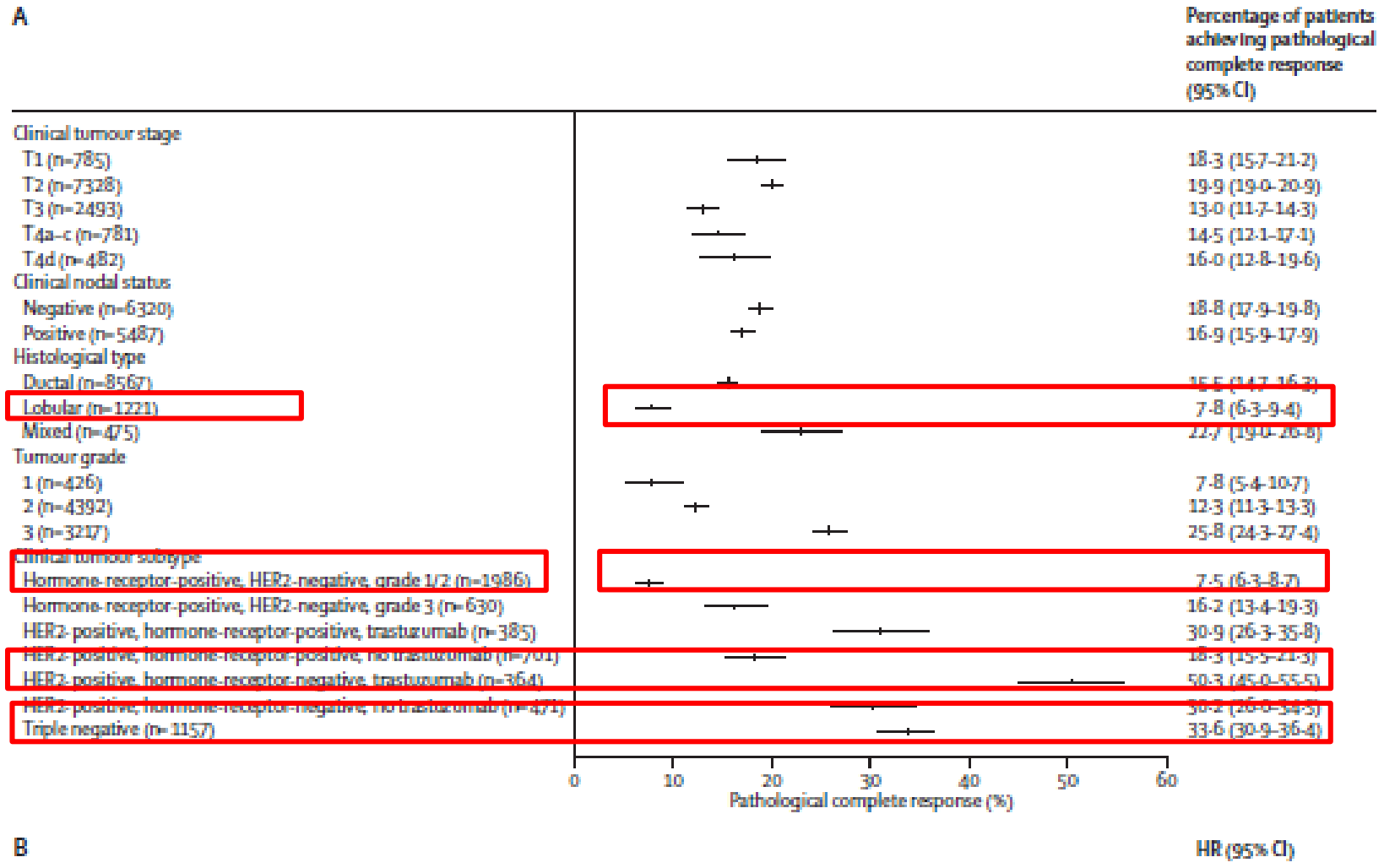
Neoadjuvant treatment

Table 2 Summary of the reported discordant ER, PR, and Her-2/neu cases post-neoadjuvant therapy

Literature review	Methods	ER discordance	PR discordance	c-erb-2 (Her-2/neu) discordance	Comment
Adams et al. [38]	IHC	2/26 (7.7 %)	4/26 (15.4 %)	6/26 (23.1 %)	Post-NAC on excision
Bogina et al. [8]	IHC	2/36 (5.5 %)	12/36 (33.3 %)		Post-CT and HT on excision
		0/25 (0 %)	2/25 (8.0 %)		Post-CT on excision
		1/24 (4.1 %)	6/24 (25.0 %)		Post-HT on excision
D'Alfonso et al. [39]	IHC/FISH	–	–	14/15 (93.0 %)	Post-NAC on excision
Idirisinghe et al. [12]	IHC	9/49 (18.4 %)	22/41 (53.7 %)	–	LR post-treatment
Kasami et al. [36]	IHC/FISH	19/173 (11.0 %)	27/173 (15.6 %)	Unchanged	Post-NAC on excision
Li et al. [37]	IHC	1.7 % ($n = 220$)	2.2 % ($n = 220$)	Unchanged	Post-NAC on excision
Nomura et al. [18]	DCA	7/15 (47 %)	6/6 (100 %)	–	LR post-treatment
Quddus et al. [59]	IHC	–	–	5/39 (12.5 %)	Post-NAC on excision
Rosen et al. [14]	DCA	6/29 (20.7 %)	ND	ND	LR post-treatment

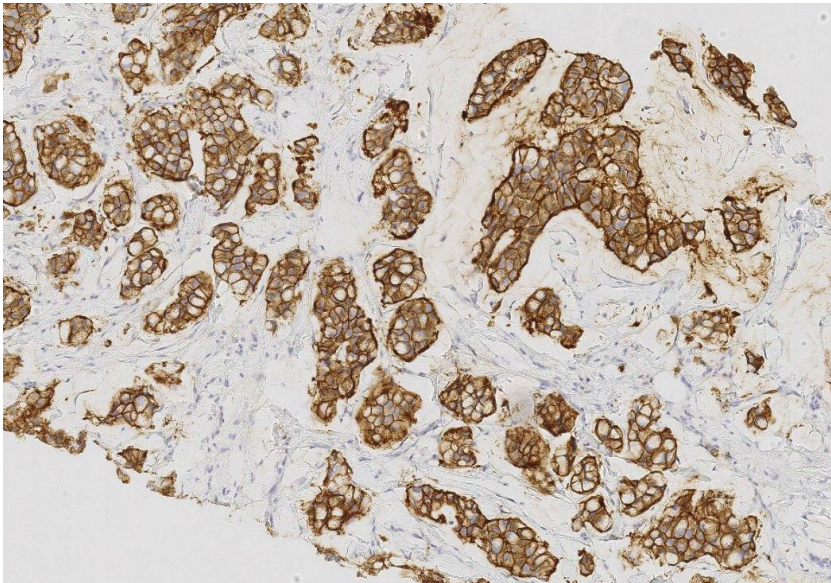
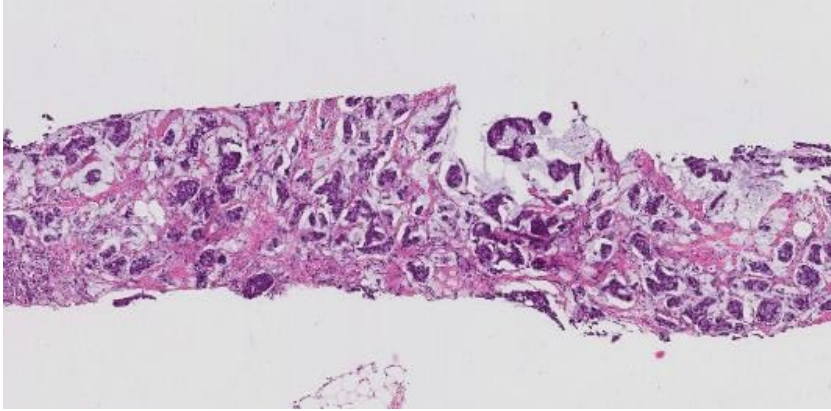
ER estrogen receptor, *PR* progesterone receptor, *Her-2/neu* epidermal growth factor receptor-2 (c-erb-2), *LR* local recurrence, *NAC* neoadjuvant chemotherapy, *IHC* immunohistochemistry, *FISH* fluorescent in situ hybridization, *DCA* dextran–charcoal assay, *HT* hormone therapy, *CT* chemotherapy

Tumor characteristics and association with pCR



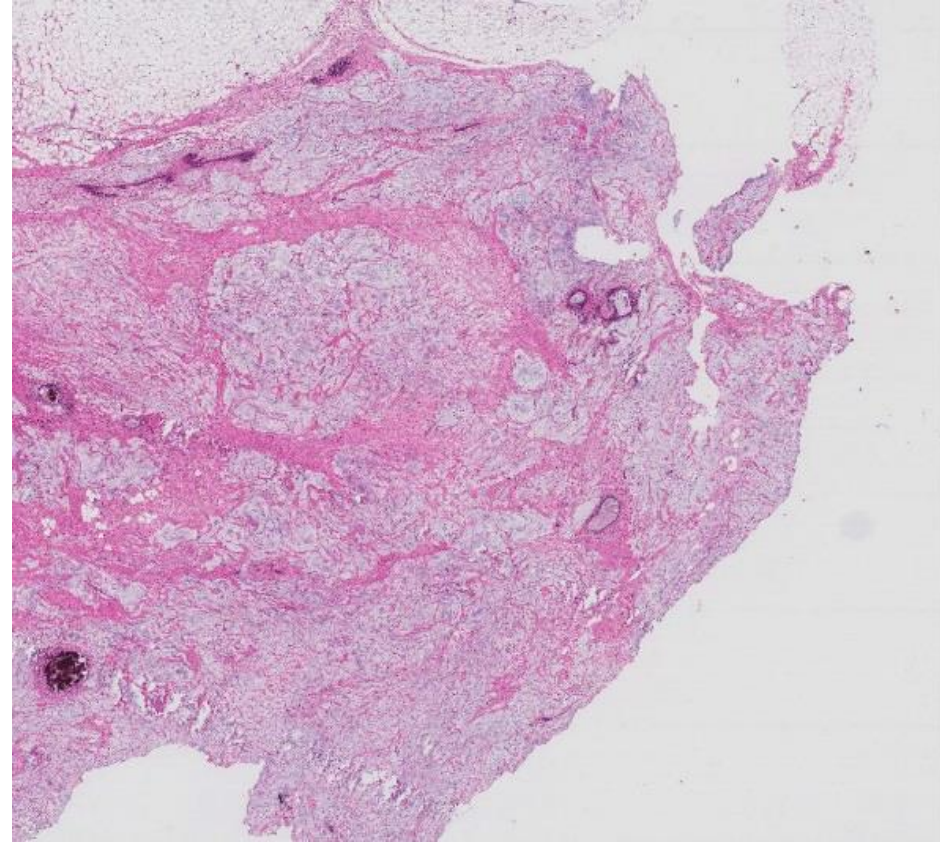
pCR

Core needle biopsy

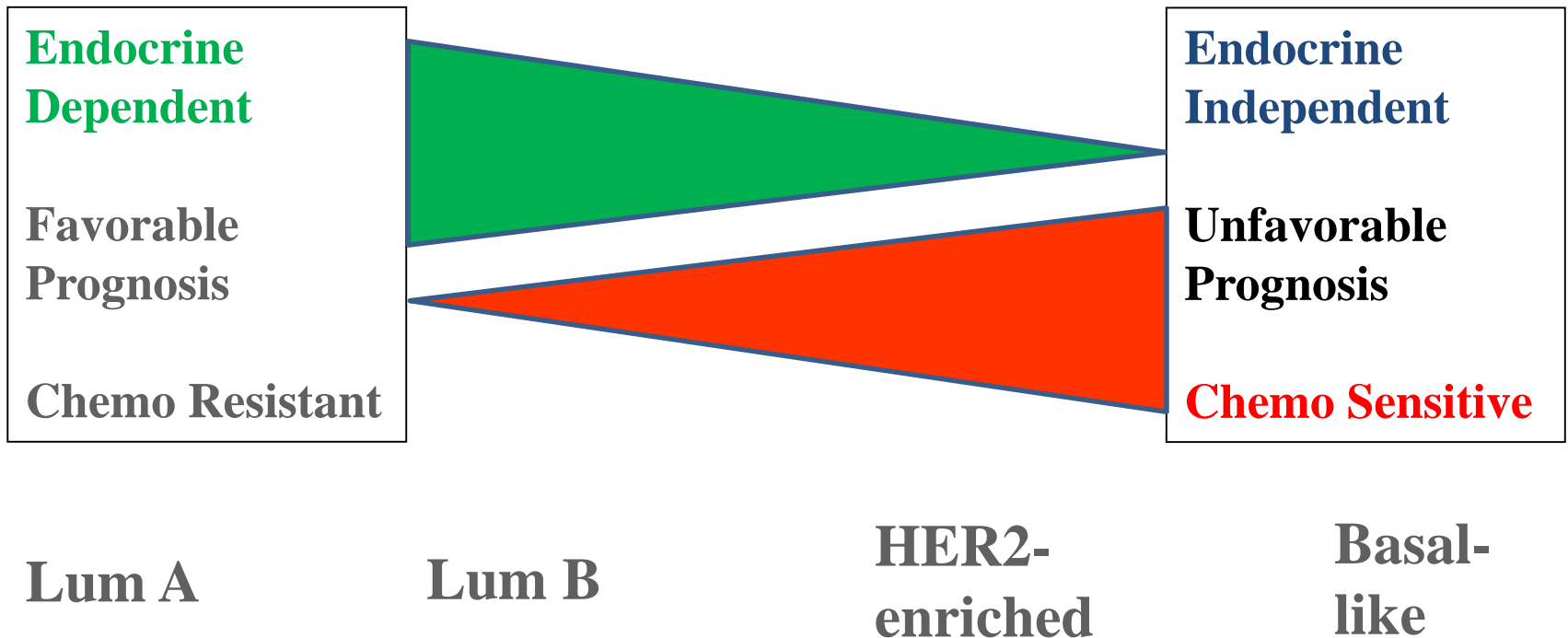


HER2 IHC

Post treatment - surgery



Breast cancer – Molecular intrinsic subtypes



Komplet patologisk respons

Sammenligning af IHC definerede grupper og molekulære subtyper

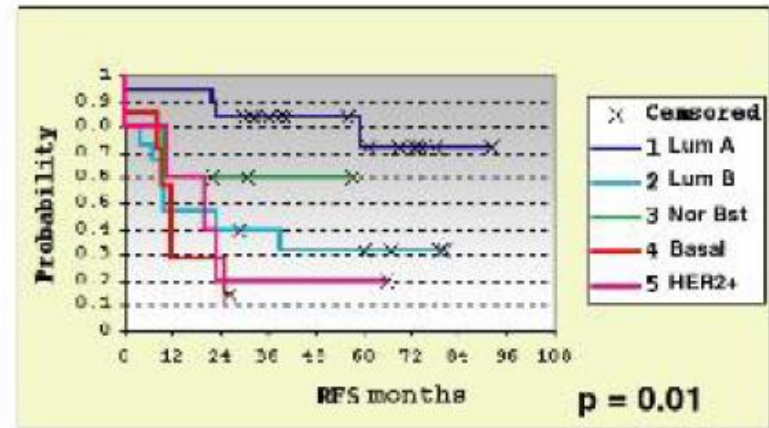
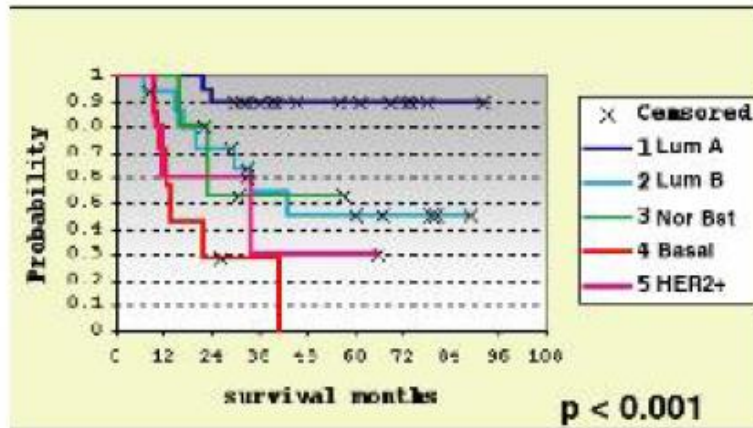
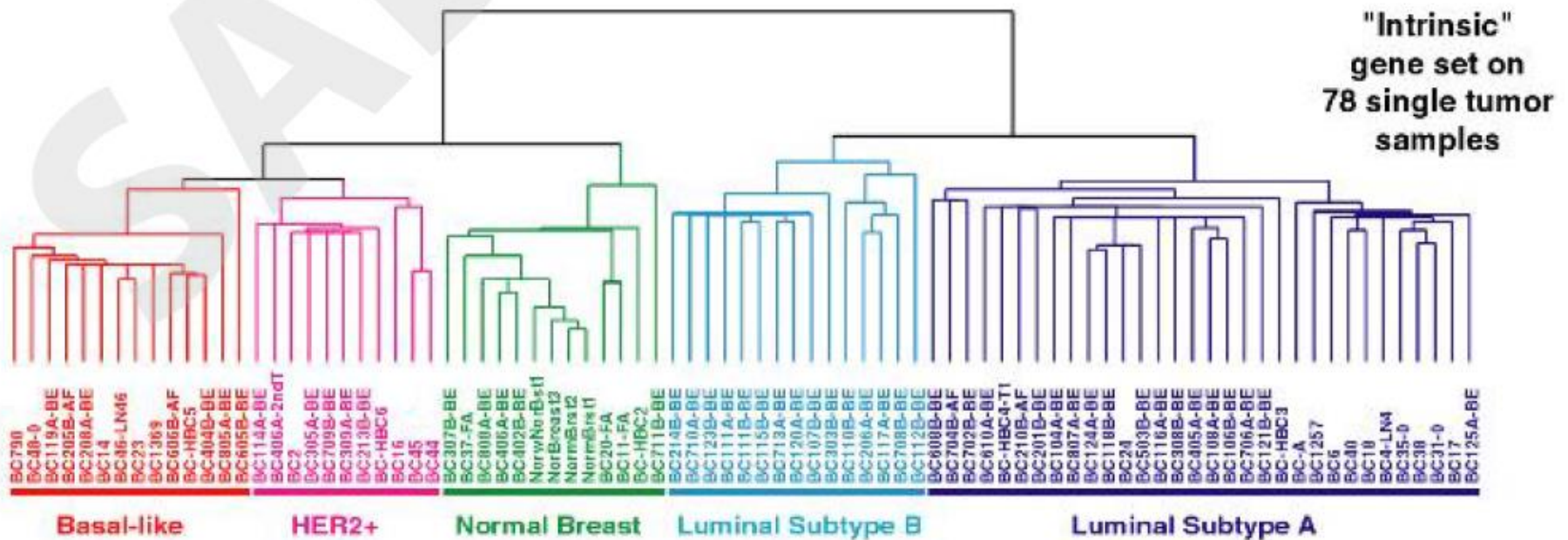
Table 4 Association of the intrinsic subtypes with chemotherapy response across the various pathology-based groups

	All patients		Luminal A		Luminal B		HER2-enriched		Basal-like		P value*
	n	pCR	n	pCR	n	pCR	n	pCR	n	pCR	
All subgroups	838	23 %	281	6 %	168	16 %	93	37 %	296	38 %	<0.001
HR ⁺ /HER2 ⁻	451	12 %	239	5 %	143	15 %	25	16 %	44	36 %	<0.001
HER2 ⁺	76	34 %	16	0 %	12	33 %	36	42 %	12	58 %	0.011
HR ⁻ /HER2 ⁻ (TN)	292	37 %	19	26 %	8	0 %	30	47 %	235	37 %	0.011

*Likelihood ratio tests: adjusting clinical features: age, clinical stage, clinical nodal status and study cohort. Hormone receptors status and HER2 status were also included in "all subgroups"

pCR, Pathological complete response; ER, Estrogen receptor; PR, Progesterone receptor

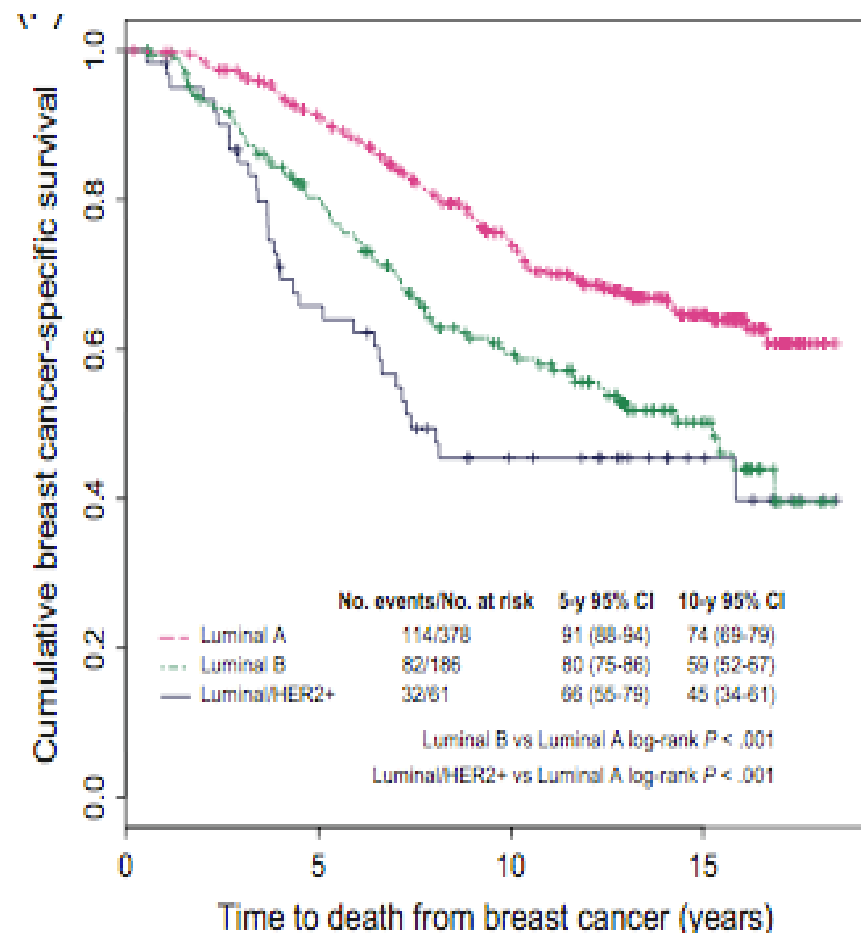
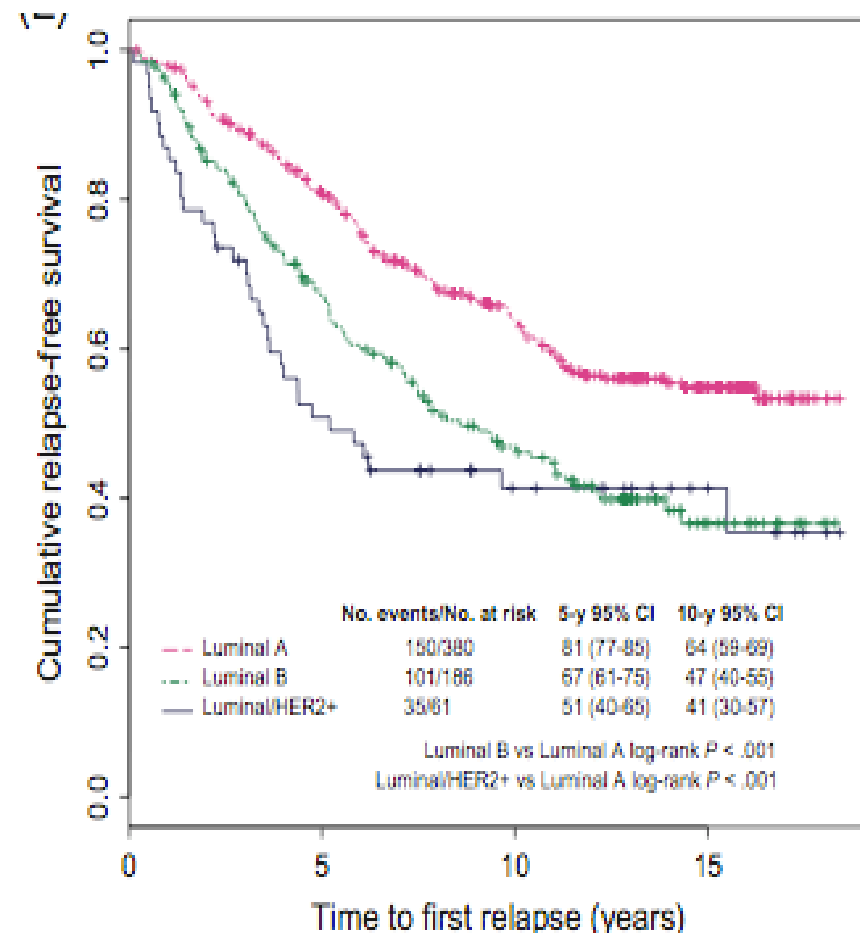
Molecular Intrinsic Subtypes 'Invasive Ductal Carcinoma'



Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

J Natl Cancer Inst 2009;101:736-750



St Gallen International Expert Consensus 2015

Annals of Oncology 00: 1–14, 2015

doi:10.1093/annonc/mdv221

Table 2. Treatment-oriented classification of subgroups of breast cancer

Clinical grouping	Notes
Triple-negative	Negative ER, PgR, and HER2
Hormone receptor-negative and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-positive	ASCO/CAP guidelines
Hormone receptor-positive and HER2-negative	ER and/or PgR positive $\geq 1\%$ ^a
luminal disease as a spectrum:	
High receptor, low proliferation, low tumor burden (luminal A-like)	Multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and clearly low Ki-67 ^b . Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).
Intermediate	Multiparameter molecular marker 'intermediate' if available ^c . Uncertainty persists about degree of risk and responsiveness to endocrine and cytotoxic therapies.
Low receptor, high proliferation, high tumor burden (luminal B-like)	Multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR with clearly high Ki-67 ^b . More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger T size (T3).

^aER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

^bKi-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.

^cNot all multiparameter molecular marker tests report an intermediate score.

COMMENTARY

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

Mitch Dowsett

N. Lynn Henry

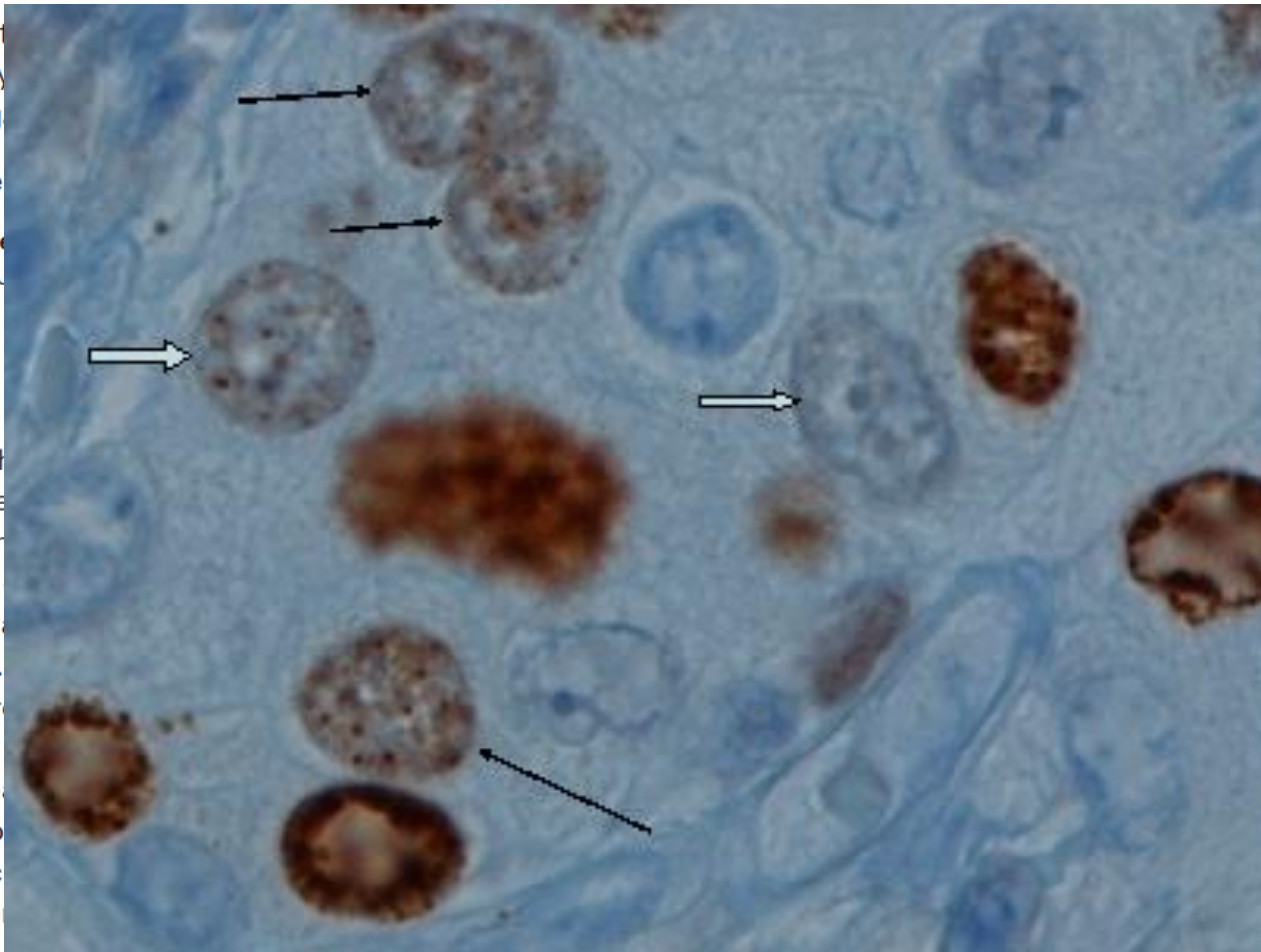
Meredith Regan

Manuscript received

Correspondence

London SW3 6JJ

Uncontrolled
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samples. Potent
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texts. On Mar
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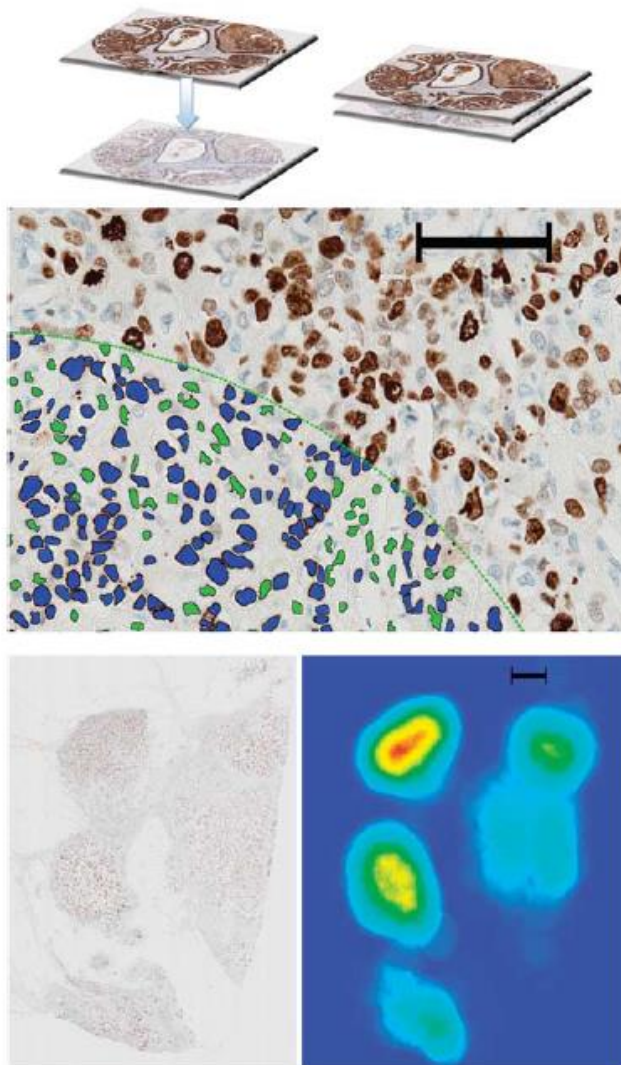
F. Hayes

Cancer Centre, Fulham Rd,

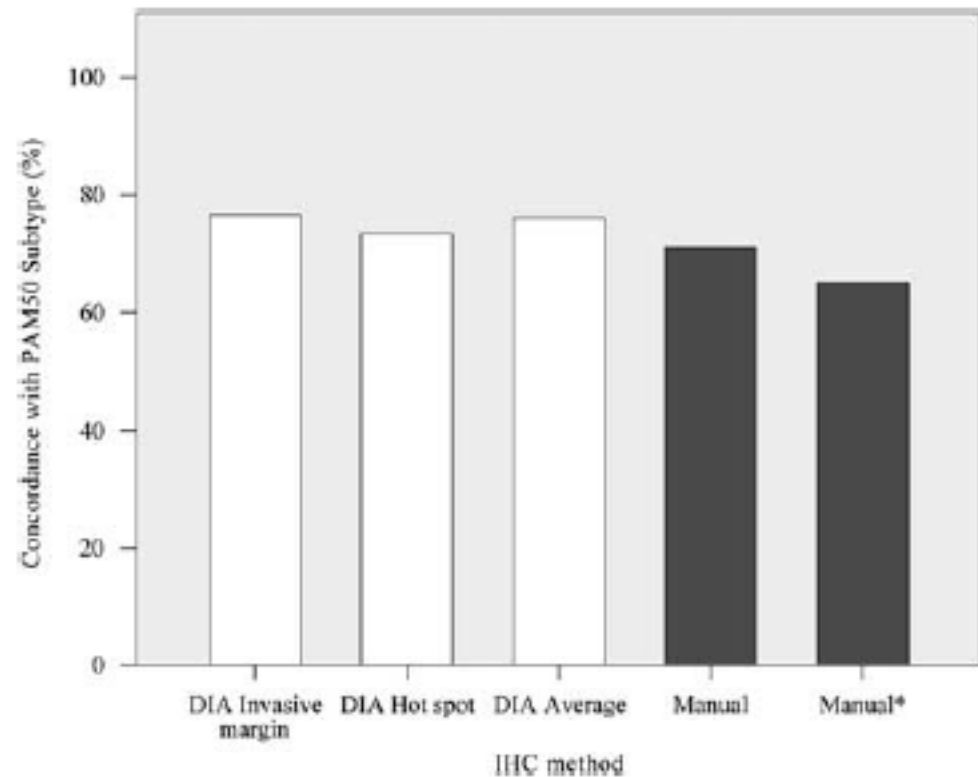
the proportion of cells
ration between tumor
otherapy or endocrine
ent efficacy in samples
ngly, Ki67 is measured
sometimes for clinical
7 in each of these con-
nent of Ki67 and in the
onal Group and North
Comprehensive recom-
mulated based on cur-
ology, create greater
in clinical practice.

Digital image analysis outperforms manual biomarker assessment in breast cancer

Gustav Stålhammar^{1,2}, Nelson Fuentes Martinez^{1,3}, Michael Lippert⁴, Nicholas P Tobin⁵, Ida Mølholm^{4,6}, Lorand Kis⁷, Gustaf Rosin¹, Mattias Rantalainen⁸, Lars Pedersen⁴, Jonas Bergh^{1,5,9}, Michael Grunkin⁴ and Johan Hartman^{1,5,7}



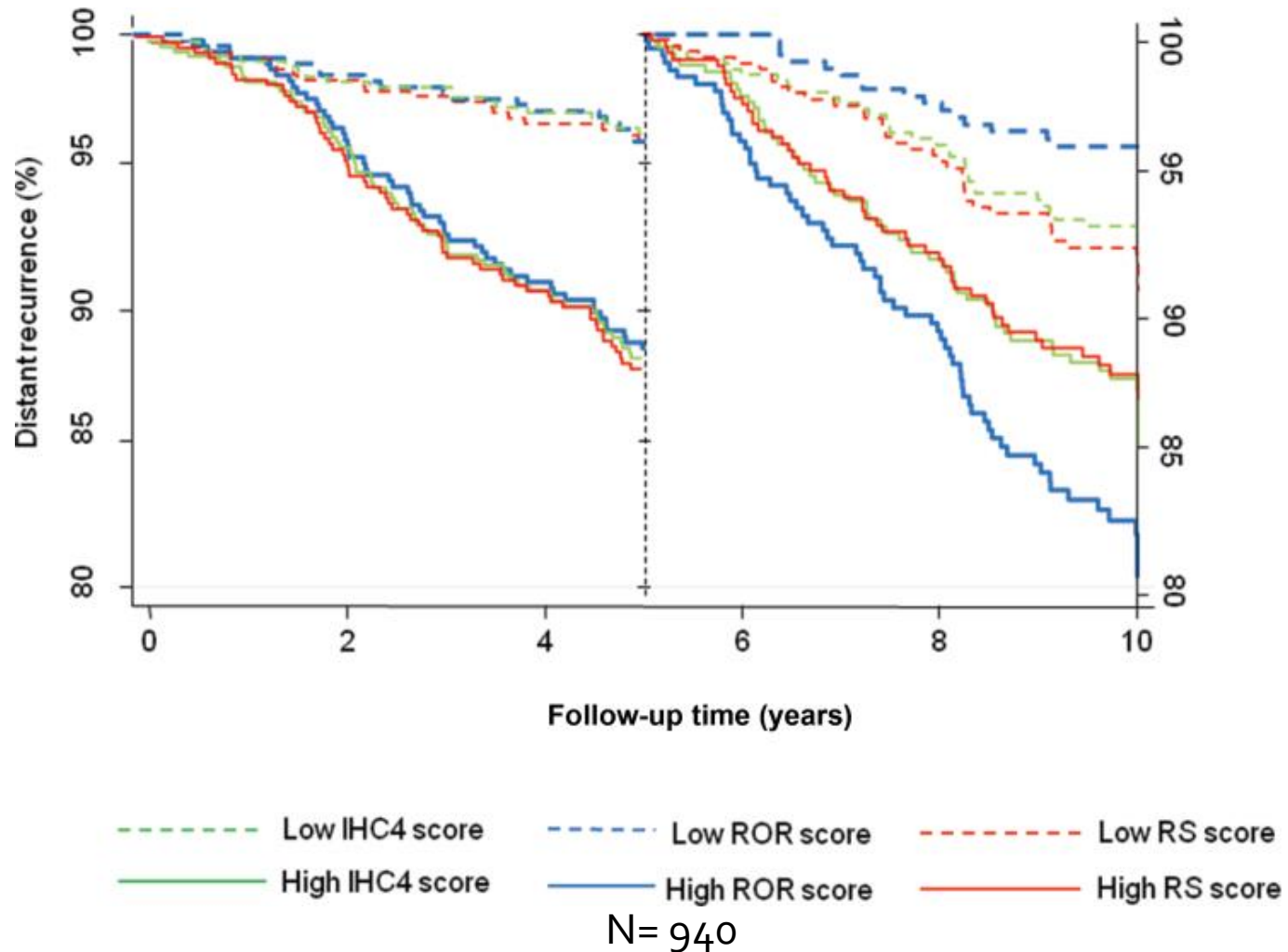
Concordance with PAM50



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Gene profile predicts late recurrence

J Natl Cancer Inst. 2013 Oct 2;105(19):12.



Available genomic tests

Table 1. Different genomic tests that are currently available to refine prognosis of patients with ER-positive HER2-negative primary breast cancer

	Oncotype Dx	MammaPrint	PAM50 ROR	EndoPredict	Breast Cancer Index (BCI)	Genomic grade
Has it been retrospectively validated on prospective phase III trials?	B-20 ATAC S-8814	x	ATAC ABCSG8	ABCSG6 ABCSG8 GEICAM 9906	ATAC Stockholm trial	BIG 1–98
Can it predict early recurrence (0–5 years)?	✓	✓	✓	✓	✓	✓
Can it predict late recurrence (after 5 years)?	x	?	✓ (superior to Oncotype Dx)	✓	✓ (superior to Oncotype Dx)	?
Can it be tested on FFPE tissue?	✓	✓ ^a	✓	✓	✓	✓
Can the test be decentralized with established reproducibility data?	x	x	✓	✓	x	x
Is it subjected to a randomized prospective trial to demonstrate clinical utility?	TailorX RxPonder	MINDACT	x	x	x	ASTER70s

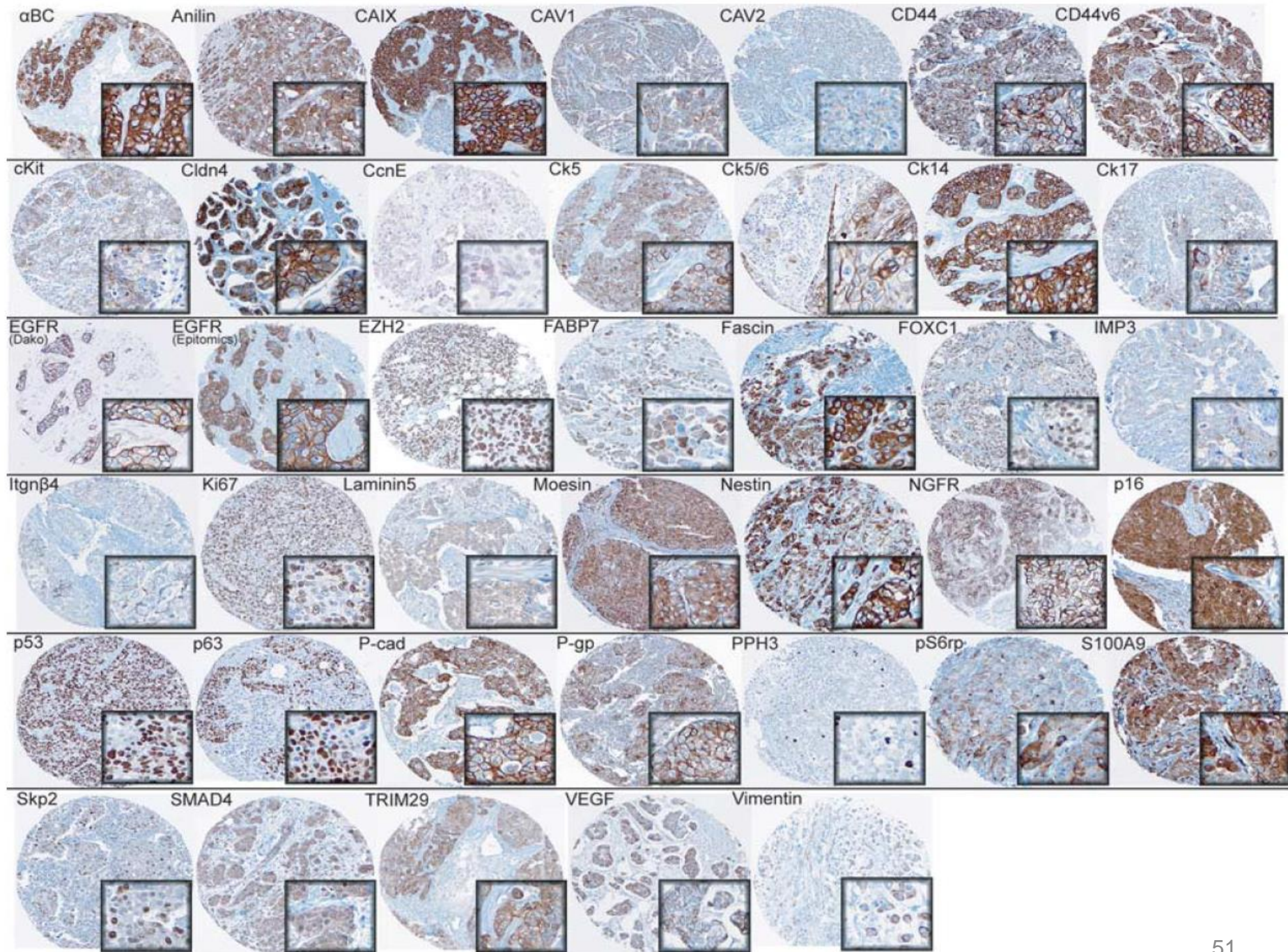
^aTest subjected to prospective validation is on frozen tissue.
FFPE, formalin-fixed paraffin-embedded.

A survey for IHC markers for basal like breast cancer against a gene expression gold standad

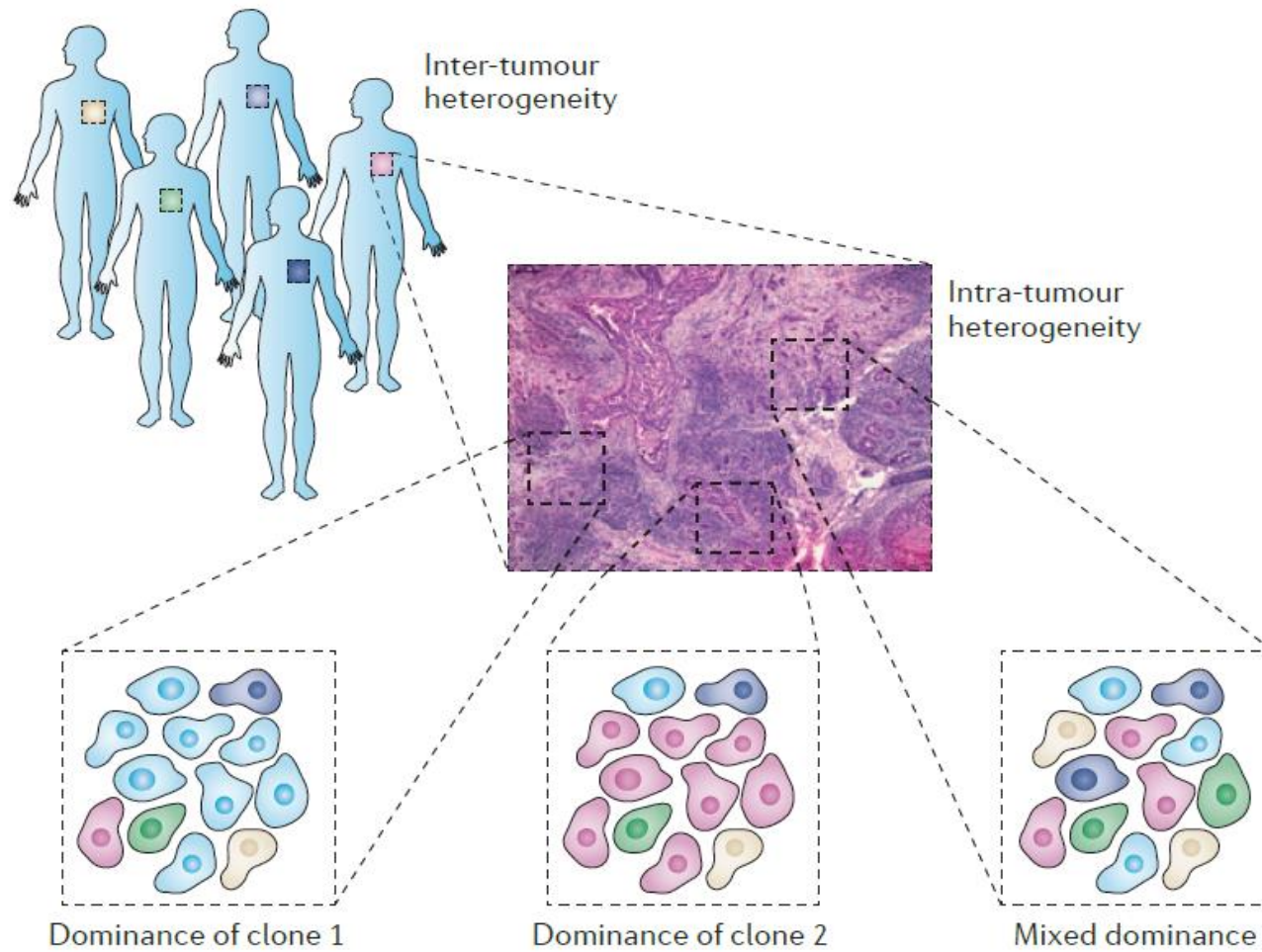
Won J et al.

Table 2 Test characteristics of statistically significant basal-like breast cancer biomarkers after correction for multiple comparisons—arranged by odds ratio (OR)

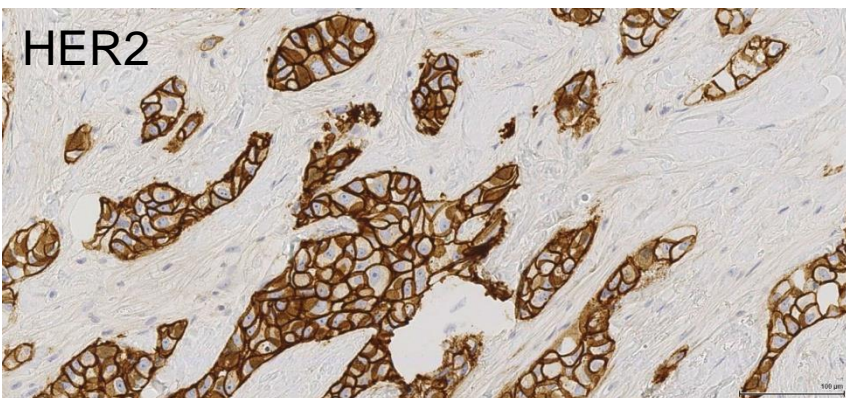
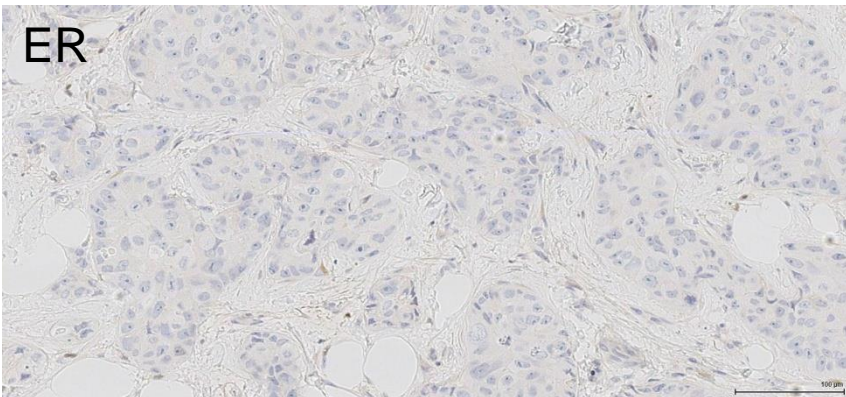
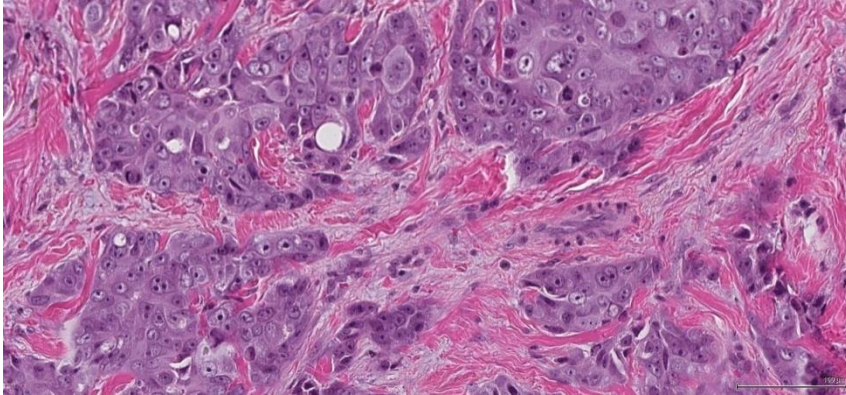
<i>Biomarker</i>	<i>Sensitivity (95% CI)</i>	<i>Specificity (95% CI)</i>	<i>OR (95% CI)</i>	<i>Raw P-value</i>
INPP4B negative	61.1 (43.8–75.9)	98.6 (91.5–100)	108.4 (13.5–872.0)	1.7E – 12
Nestin	54.1 (37.2–70.0)	95.8 (88.2–98.7)	26.7 (7.1–100.3)	1.9E – 09
ER negative	92.1 (78.6–97.6)	67.6 (56.1–77.6)	24.3 (6. 8–87.0)	2.1E – 09
CK5	70.6 (52.8–84.2)	90.3 (80.3–96.2)	22.4 (7.3–68.6)	7.4E – 10
cKit	42.4 (25.6–59.4)	96.8 (88.2–100)	22.1 (4.6–106.1)	2.9E – 06
p16	78.8 (61.3–90.5)	83.9 (72.7–91.7)	19.3 (6.6–56.6)	2.0E – 09
Fascin	57.9 (41.5–73.0)	92.9 (84.4–97.2)	17.9 (5.9–54.5)	6.0E – 09
PPH3	91.7 (77.8–97.5)	58.0 (45.9–69.2)	15.2 (4.2–54.3)	3.5E – 07
Moesin	71.4 (53.3–84.4)	84.6 (73.7–91.9)	13.8 (5.1–37.2)	2.1E – 08
CK17	50.0 (31.0–65.7)	91.9 (82.5–96.9)	11.4 (3.6–35.9)	3.7E – 06
ki67	92.1 (78.4–97.7)	49.3 (37.8–60.8)	11.4 (3.2–40.2)	7.3E – 06
PR negative	92.1 (78.6–97.6)	46.6 (35.0–58.2)	10.2 (2.9–36.1)	3.5E – 05
TRIM29	71.0 (51.9–85.2)	78.8 (67.2–87.3)	9.1 (3.4–24.1)	2.2E – 06
α-B-crystallin	41.2 (25.0–58.3)	92.3 (83.3–97.0)	8.4 (2.7–26.3)	5.9E – 05
S100A9	62.2 (45.5–76.9)	82.4 (71.4–90.2)	7.7 (3.1–19.1)	3.8E – 06
CK5/6	50.0 (32.6–64.9)	87.7 (78.1–93.9)	7.1 (2.8–18.3)	1.4E – 05
Skp2	60.6 (42.4–76.0)	81.0 (69.4–89.1)	6.5 (2.6–16.7)	4.1E – 05
EGFR (Epitomics)	51.4 (34.3–67.6)	85.7 (75.4–92.4)	6.4 (2.5–16.3)	5.0E – 05
P-cadherin	77.8 (60.7–89.2)	60.9 (48.5–71.6)	5.4 (2.2–13. 7)	1.7E – 04
Claudin 4	63.9 (46.4–78.6)	72.3 (60.0–82.3)	4.6 (1.9–11.0)	3.9E – 04
Cyclin E	69.4 (51.9–82.9)	66.2 (54.4–76.4)	4.5 (1.9–10.6)	4.7E – 04
p53	52.8 (36.1–69.0)	79.7 (68.7–88.1)	4.4 (1.8–10.6)	6.6E – 04
CK14	27.0 (13.9–43.2)	100	—	1.1E – 05
IMP3	25.0 (11.8–40.6)	100	—	3.1E – 05
NGFR	22.2 (10.3–38.1)	100	—	1.3E – 04



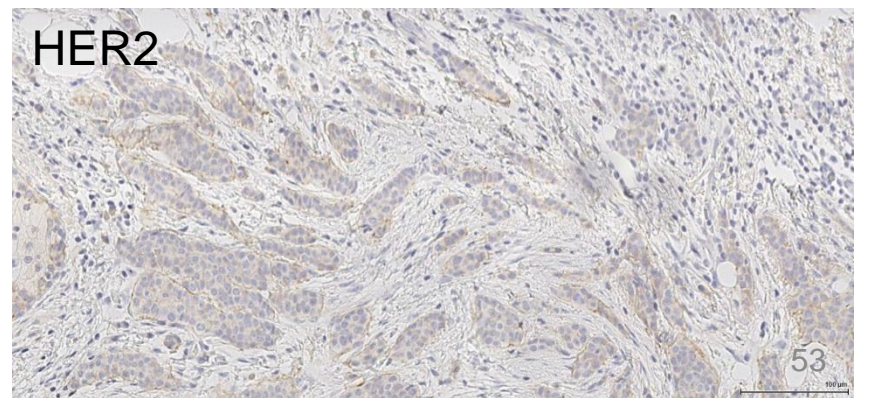
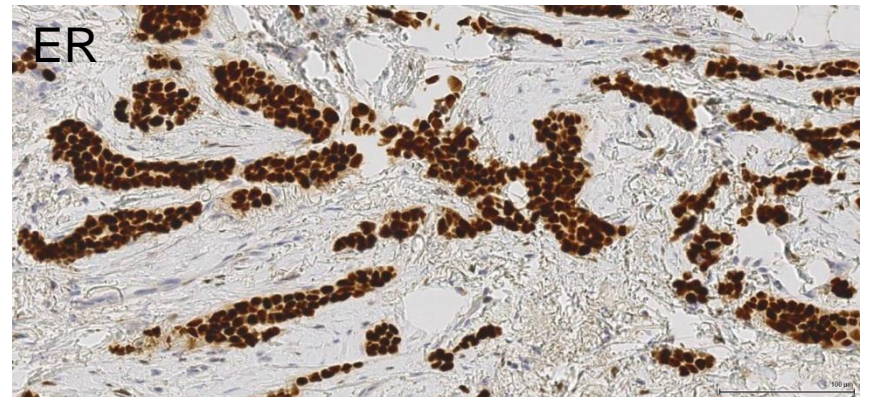
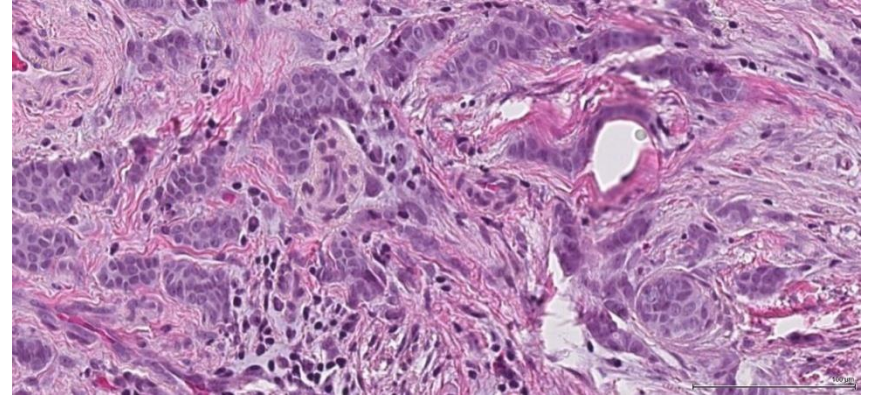
Tumor heterogeneity



Tumour 1



Tumour 2



Analysis of ER and HER2 in metastatic lesions

Author/ Publication year/ Reference	Number analyzed (ER/HER2/ TOP2A)	Location of biopsy	ER* (%)	HER2* (%)	TOP2A* (%)	Comment
Wilking et al (2011) (66)	151	LR+distant ⁵	-	10%	-	No re-analysis ¹
Fabi et al (2011) (67)	137	3/4 LR	-	10%	-	
Amir et al (2010) (51)	258	LR+distant	13%	5%	-	Two prospective studies, pooled
Locatelli et al (2010) (49)	255/167	Distant ⁶	16%	13%	-	No re-analysis ¹
Lindstrom et al (2010) (50)	477/108	-	33%	10%	-	No re-analysis ¹ , IHC+ICC+biochemical
Karlsson et al (2010) (62)	486	-	35%	-	-	No re-analysis ¹ , IHC+ICC+biochemical
Lower et al (2009) (65)	382	-	-	33%	-	No re-analysis ¹ , IHC only ³
Simmons et al (2009) (54)	25	Distant	12%	8%	-	Prospective study
Broom et al (2009) (48)	62/18	-	18%	6%	-	No re-analysis ¹
Liedtke et al (2009) (56)	231	-	18%	14%	-	No re-analysis ¹
Guarneri et al (2008) (55)	75	LR+distant	22%	16%	-	Not all re-tested ⁴
MacFarlane et al (2008)(186)	160	LR+distant	28%	-	-	Total discordance (ER/PgR/HER2)
Tapia et al (2007) (68)	105	Distant ⁶	-	8%	-	IHC (prim BC), ICC (MBC), only FISH
D'Andrea et al (2007) (187)	88/76	syn LN ²	3%	4%	-	
Zidan et al (2005) (64)	58	-	-	14%	-	
Gong et al (2005) (71)	60	2/3 LR	-	3%	-	1/3 synchronous LN, IHC+ICC
Franco et al (2004) (59)	658	-	29%	-	-	A meta-analysis
Gancberg et al (2002) (69)	93/68	Distant	-	6/7%	-	By IHC (6%)/FISH (7%)
Cardoso et al (2001) (188)	370/161	syn LN	-	2%	19%	IHC (TOP2A, HER2) only
Tanner et al (2001) (70)	46/13	2/3 LR	-	0%	23%	Only TOP2A in 13 pt
Kuukasjrvi et al (1996) (57)	50	2/3 LR	24%	-	-	Cut-off: ≥ 20 % pos.
Abbreviations: LN: lymph nodes, LR: locoregional asynchronous disease (i.e. lymph node, scar, and residual breast recurrence), ICC: immunocytochemical analysis.”-“: No available information. BC: Breast Cancer, MBC: Metastatic Breast Cancer.						
*Discordance in percent; ¹ No re-analysis done, i.e. based on original pathology reports. ² Assessed on synchronous axillary nodes (i.e. lymph node involvement at diagnosis). ³ IHC 2+ scored as HER2 positive. ⁴ Did re-evaluate, but not re-test all samples. ⁵ The proportion of LR and distant unknown. ⁶ Assessed from distant metastases.						

- ER discrepancy: 12 – 29%, often with loss of receptor
- HER2 discrepancy: 6 – 20%, often with gain of HER2+

Limitations:

- Many ”pathology chart review” studies, did not re-analyse tumor samples (methodological variation)
- Prospective studies:
 - Biopsy had treatment consequence in 15-20%
 - Benign disease/other malignancies in 14%

American Society of Clinical Oncology/College of American Pathologists Guideline
Recommendations for Immunohistochemical Testing of:
Estrogen and Progesterone Receptors (Arch Pathol Lab Med. 2010;134:e48-e72) and
HER2 (ahead of print at www.jco.org on October 7, 2013) in Breast Cancer
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- Preanalytical standardization
 - Fixation
- Analytical standardization
 - Antibody/Antigen Retrieval/Detection Systems
 - Control samples
- Postanalytical standardization
 - Interpretation
 - Cut-off level
 - Internal quality control
 - Tissue / Material
 - Image Analysis
- Participation in quality assurance programs

