Immunohistochemical classification of breast tumours

Workshop in Diagnostic Immunohistochemistry

September 19th - 21th 2018

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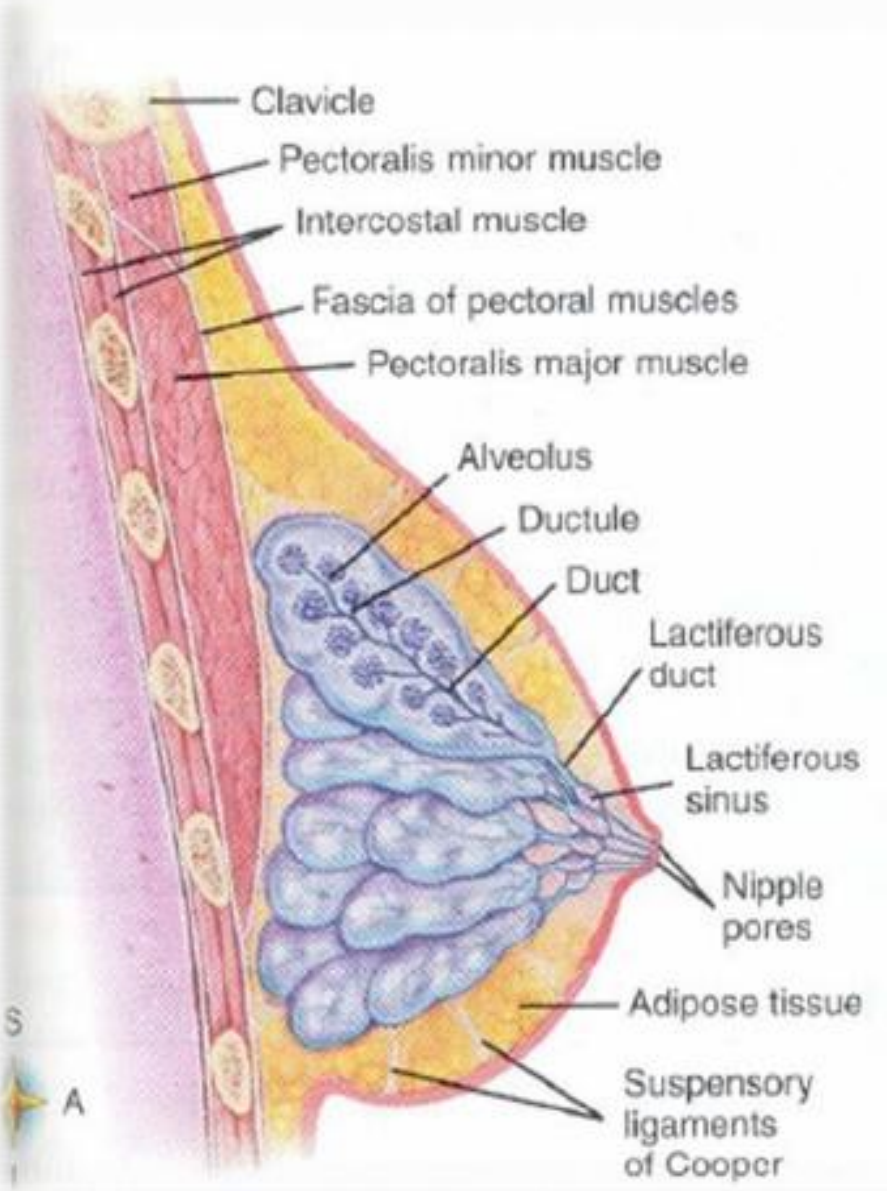
Slagelse
Denmark

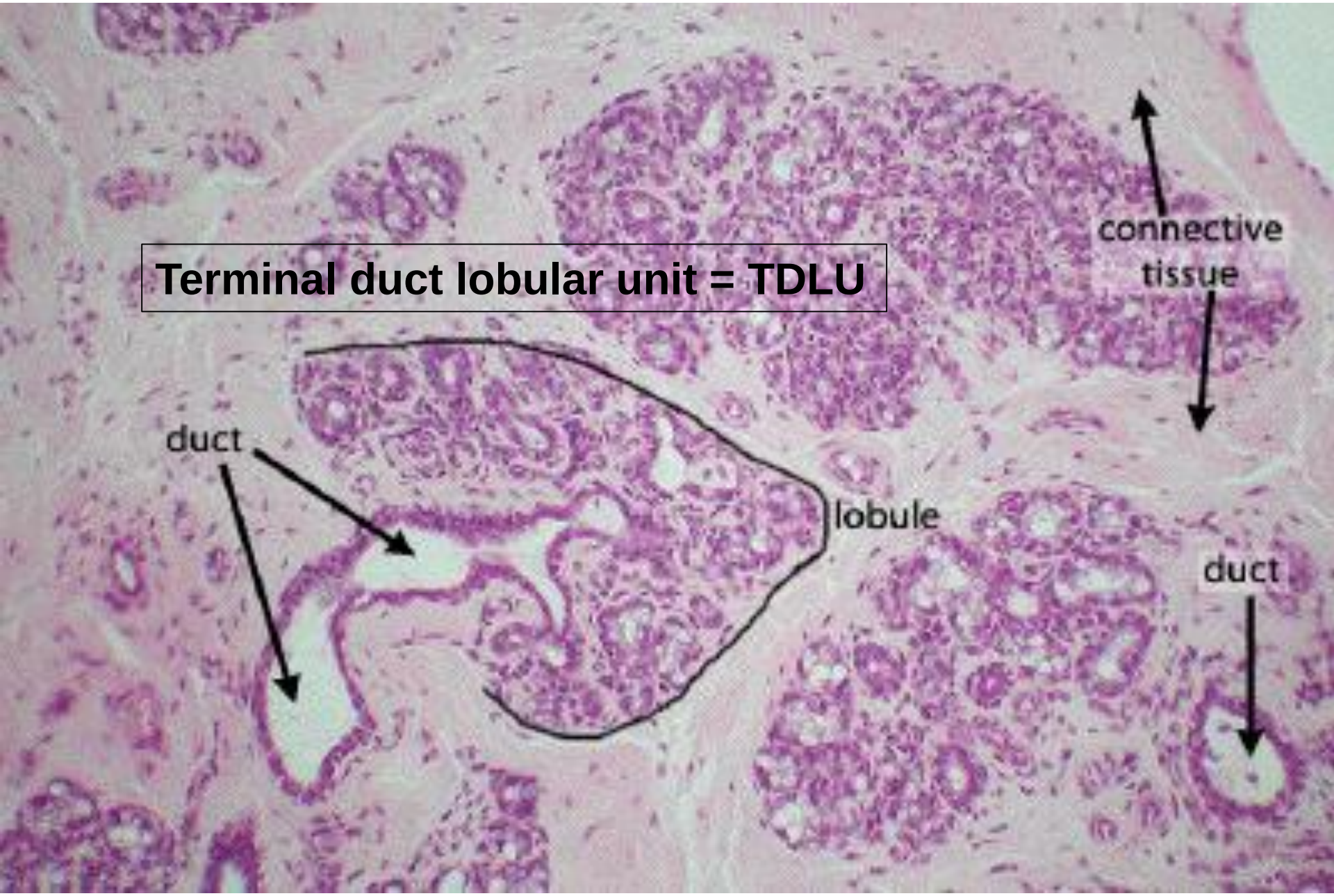
Agenda

- Immunohistochemical biomarkers for
  - Diagnostics
    - Benign Hyperplasia and Ductal Carcinoma in Situ
    - Ductal Carcinoma in Situ and Lobular Carcinoma in Situ
    - Carcinoma In Situ and Invasive Carcinoma
  - Histological subtype classification
    - Malignant breast tumors
  - Predictive/Prognostic markers
    - Estrogen Receptor
    - Progesteron Receptor
    - HER2
    - Ki67
  - Intrinsic subtype classification by surrogate IHC biomarkers?
  - Tumor heterogeneity

ANATOMY OF BREAST

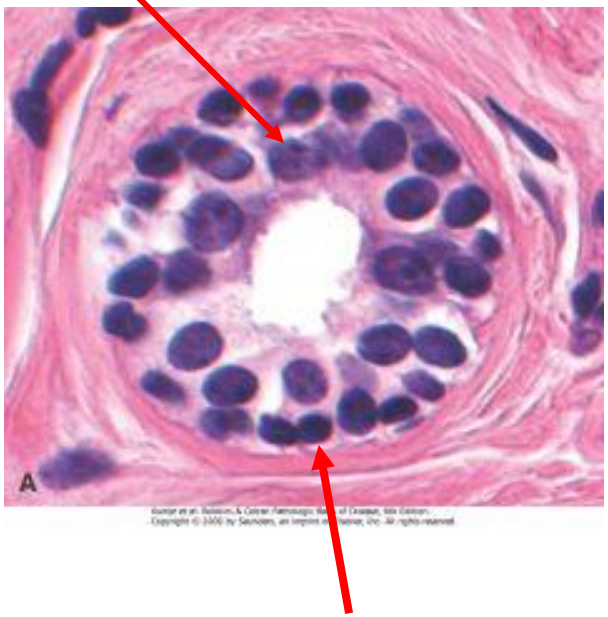
- Modified **apocrine sweat glands**.
- Breast parenchyma → 12 to 20 **lobes**.
- Within each lobe – Lactiferous duct - branches repeatedly → leads to no. of terminal ducts → each leads to a **lobule→ contains multiple acini/alveoli → *TDLU (TERMINAL DUCT + LOBULE)***
- Spaces around the lobules and ducts and between the lobes are filled with **fatty tissue, ligaments and connective tissue → *STROMA***



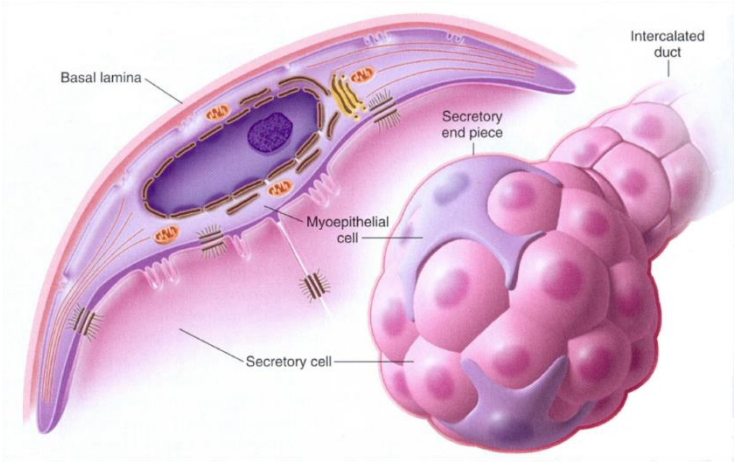

Terminal duct lobular unit = TDLU
connective tissue
duct
lobule
duct

Mammary gland
2 types of epithelial cells are present: Luminal cells and myoepithelial cells

Luminal cell

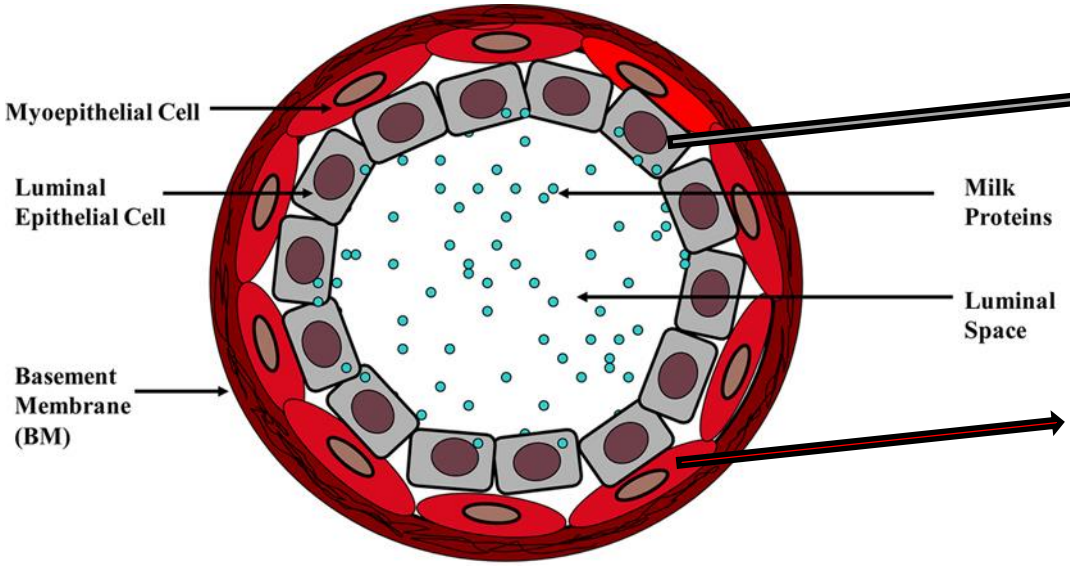


Myoepithelial cell



Myoepithelial cells with contractile function forming a meshwork that does not cover the entire basement membrane nor the entire luminal cell

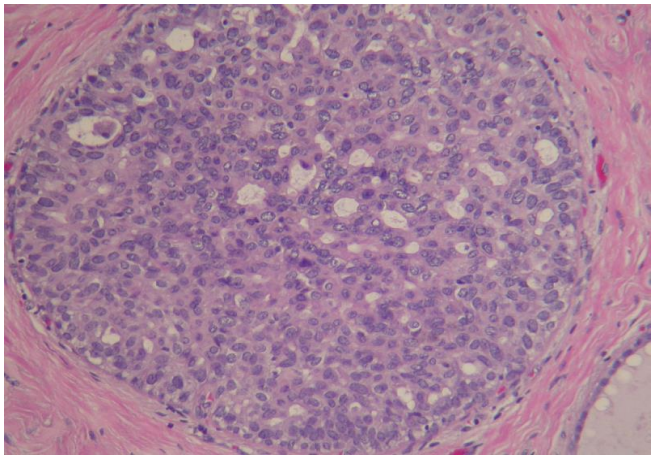
Immunohistochemical phenotype

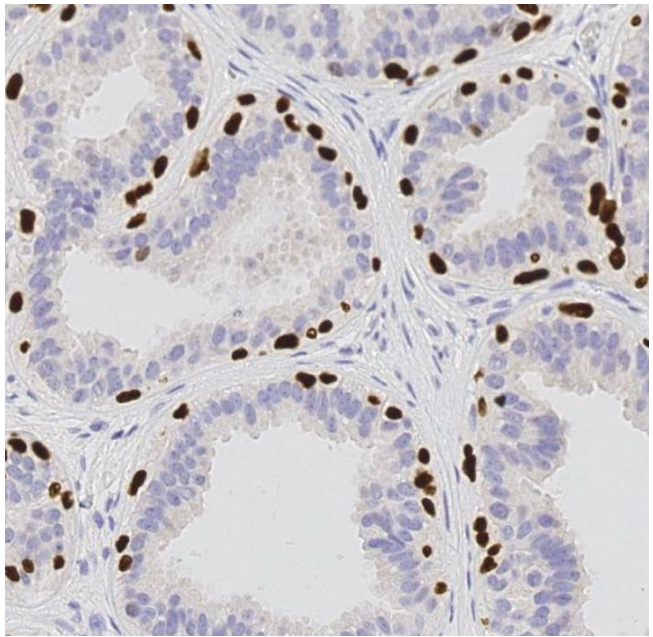


Luminal markers (LMW):
CK7, CK8, CK18, CK19

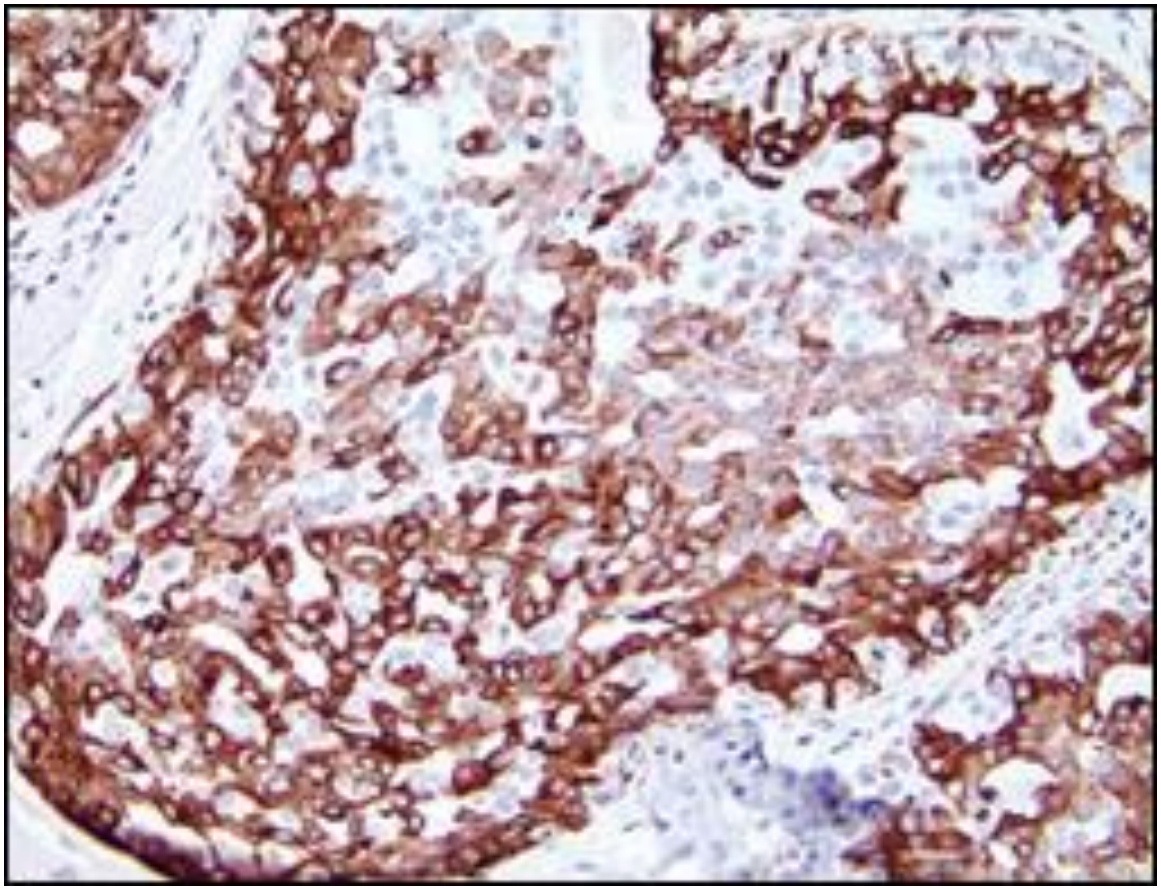
Myoepithelial markers:
Myo: p63, SMA, CD10, SMMHC
Cytokeratins (HMW): CK5, CK14, CK17

Benign hyperplasia
Positive staining for myoepitelial cells





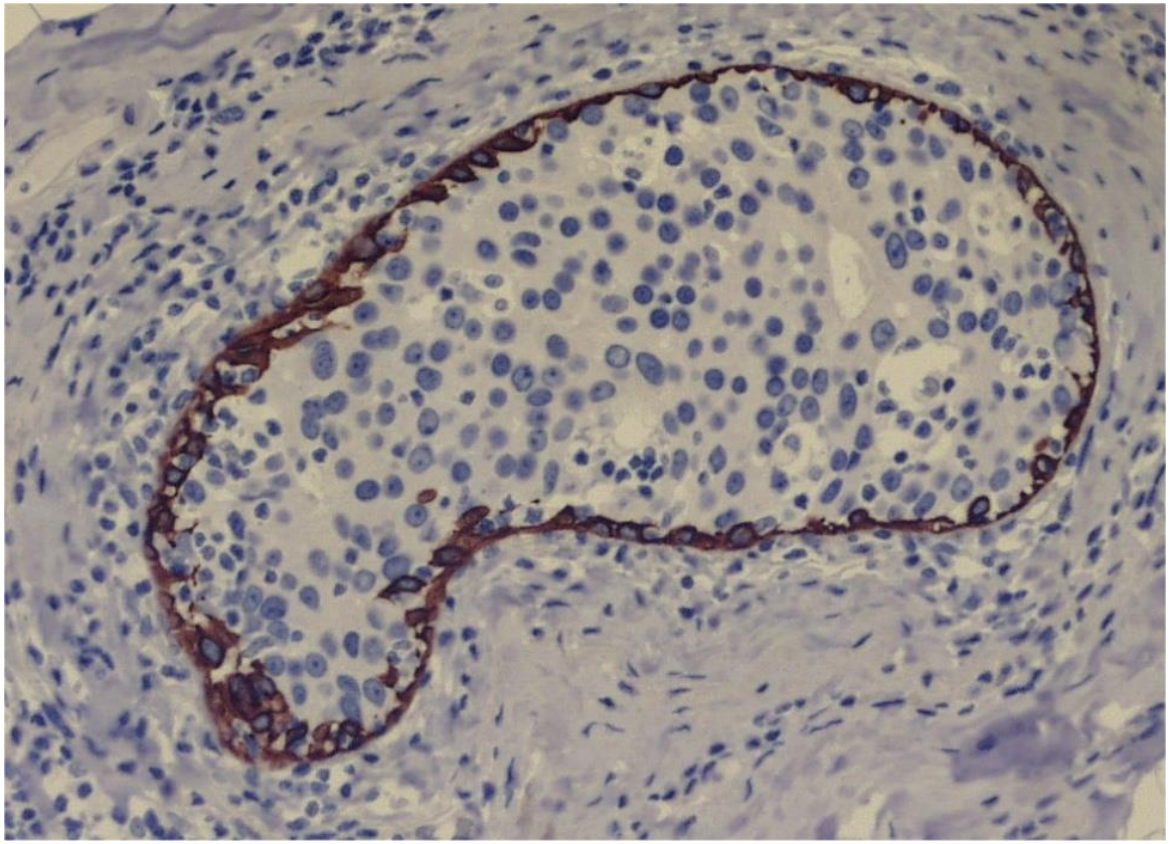
P63



CK5

Ductal Carcinoma In Situ

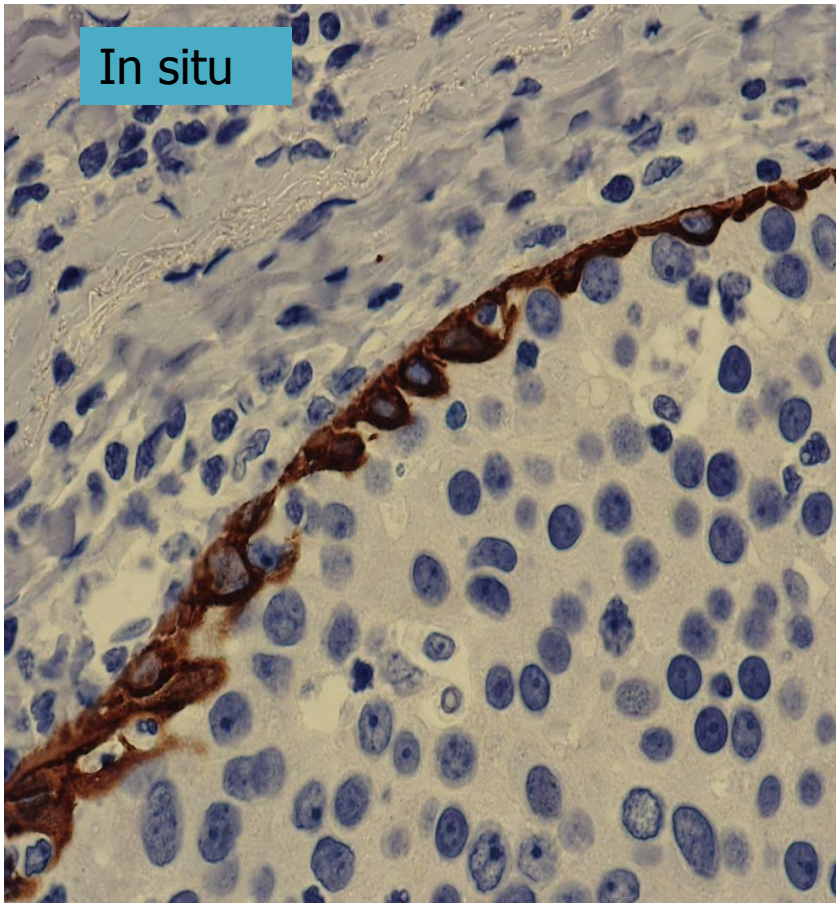
CK14 Ductal Carcinoma In Situ



Monotonous epithelial proliferation within ducts

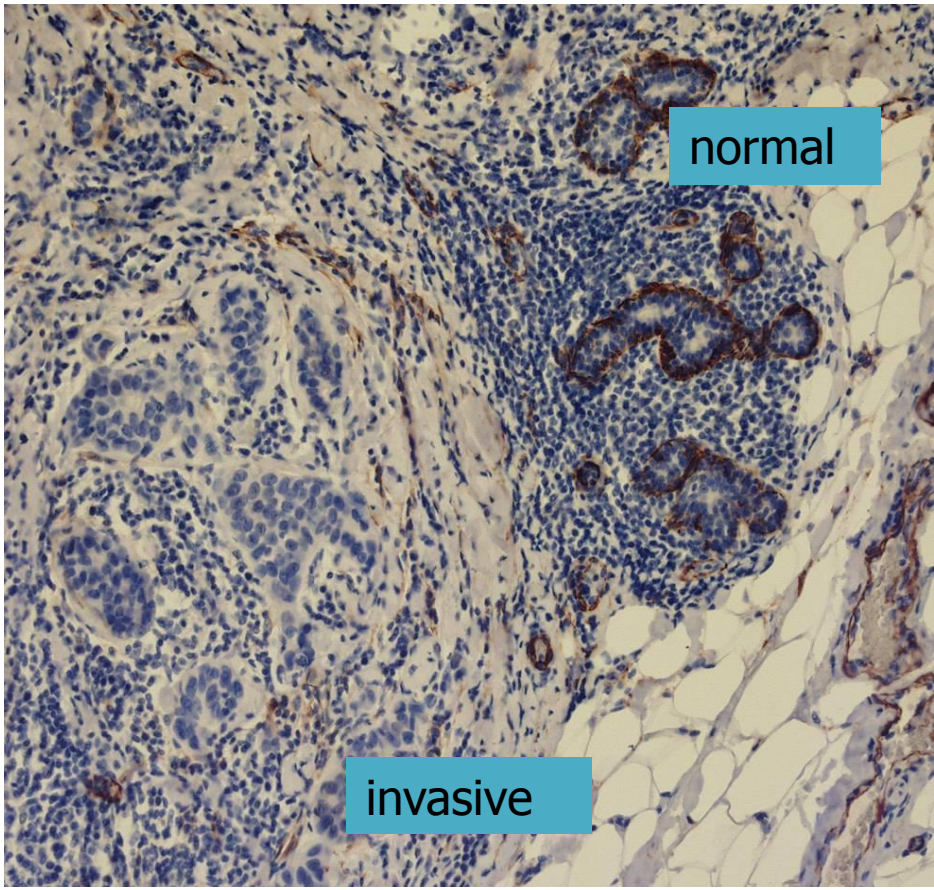
Invasive Carcinoma
i.e. SMMHC

present

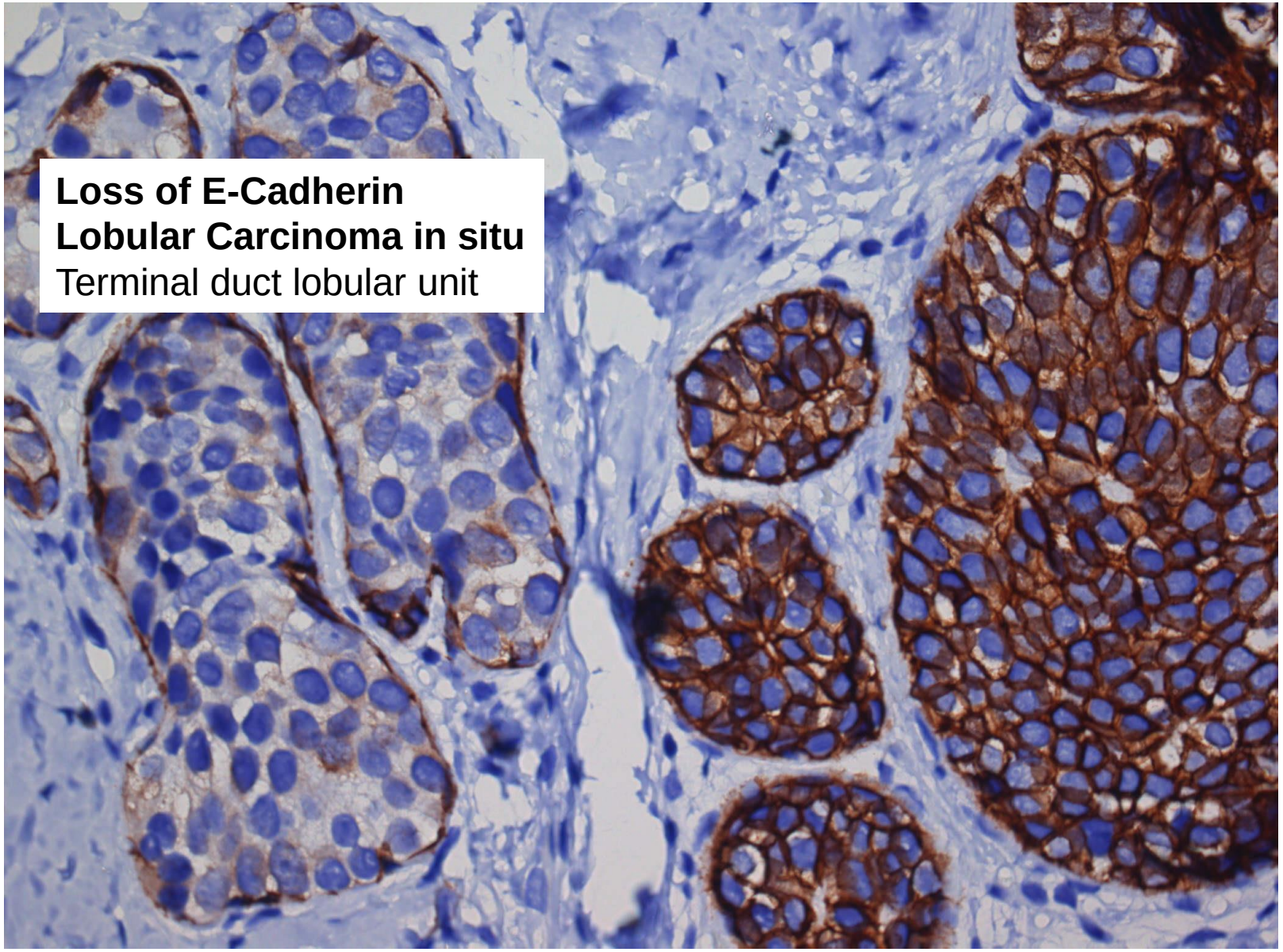


Detecting ”presence”

Not present



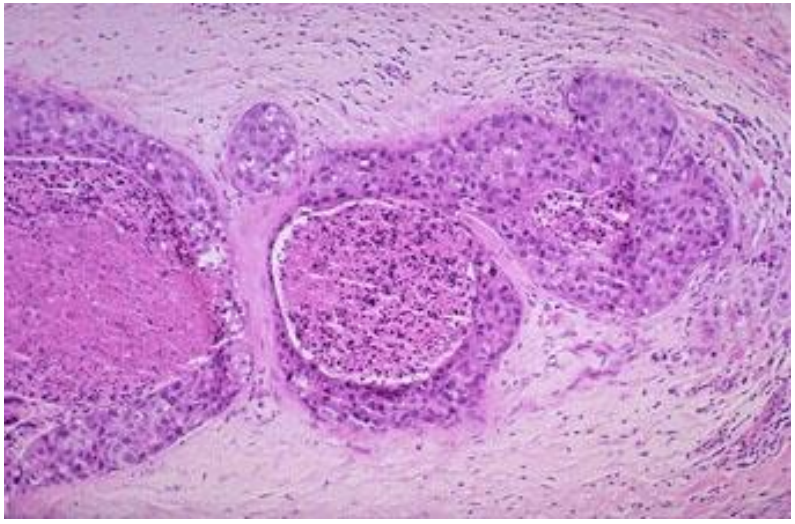
Detecting ”absence”



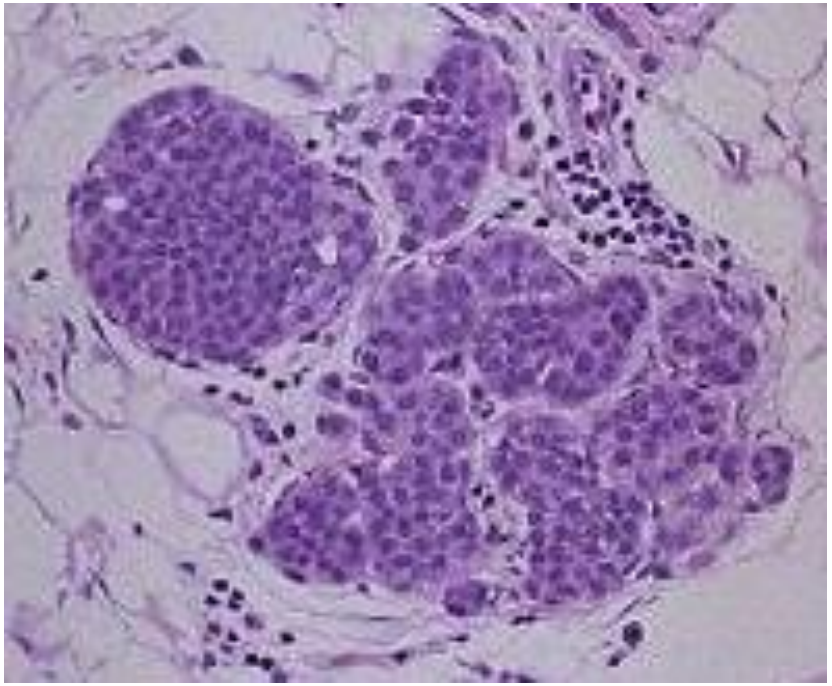
E-cadherin: Cell Adhesion Molecule

Carcinoma in situ

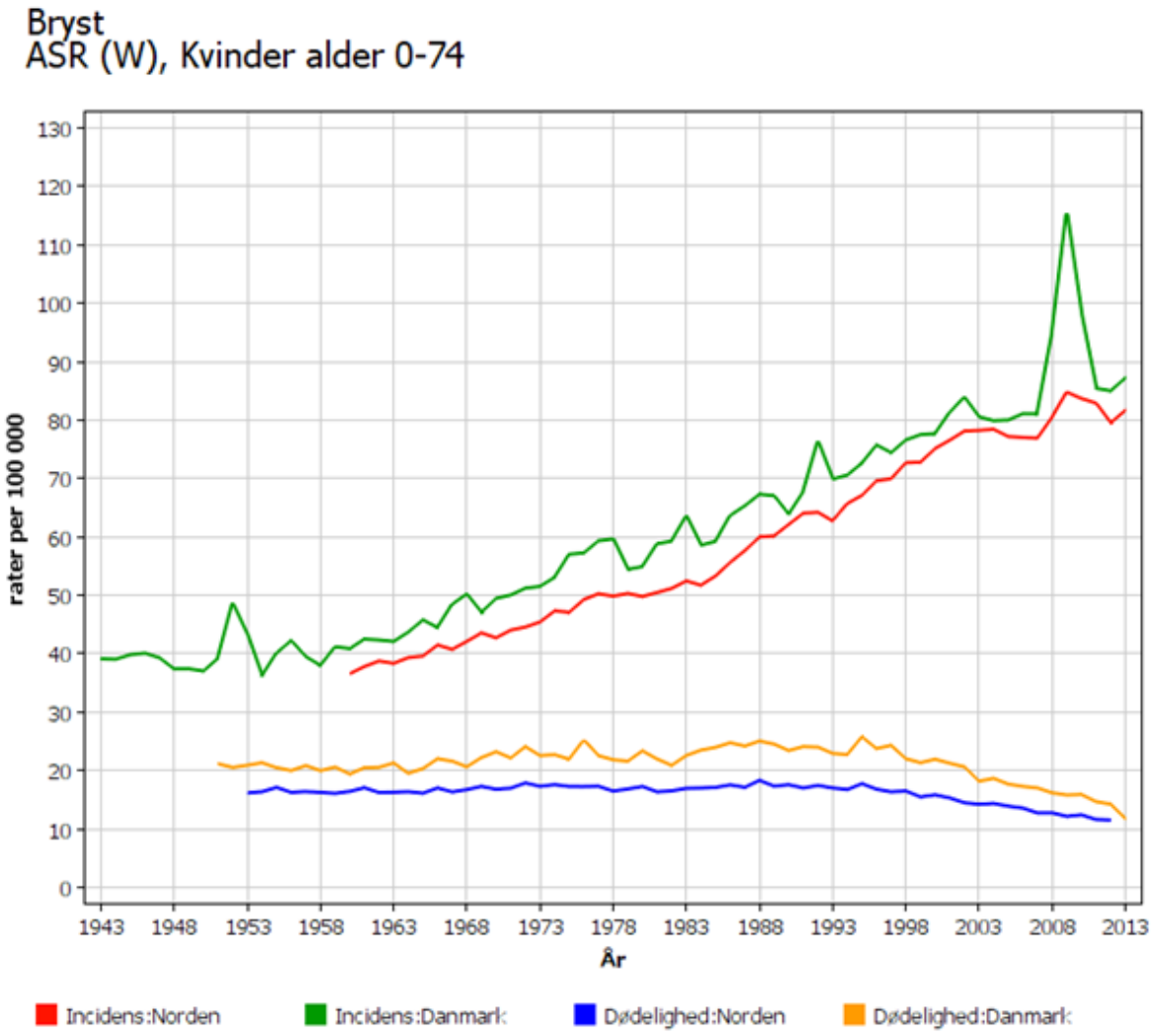
- Ductal carcinoma in situ
  - 12-15% of malignant lesions in the Danish screening population
  - Microcalcifications
  - Risk of progression to invasive carcinoma
  - Surgery with free margins
  - Radiation therapy after breast conserving surgery



- Lobular carcinoma in situ
  - Incidence 0.5 – 3.6%
  - Often incidental finding
  - Multifokal and often bilateral
  - Slowly proliferating lesions
  - Observation / screening

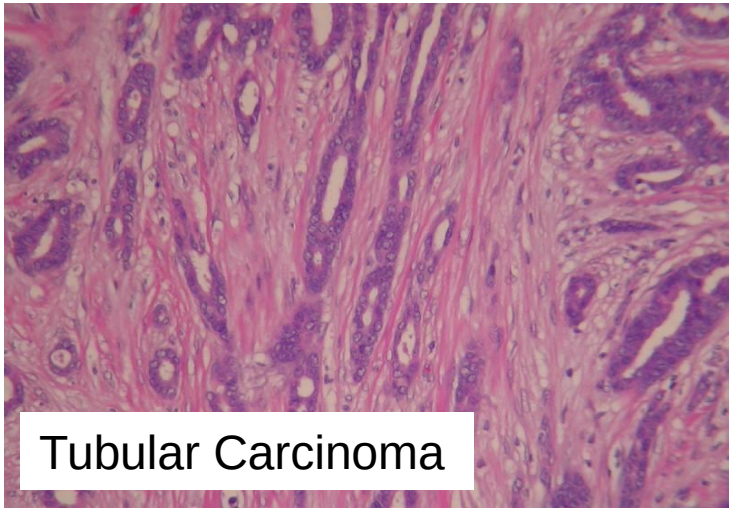


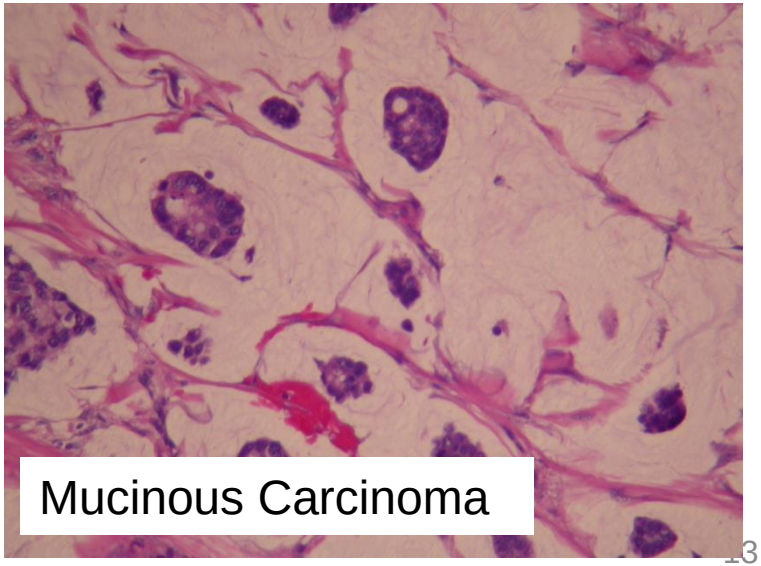
Breast cancer: Incidence and mortality Denmark



Invasive Breast Cancer Histological Subtypes

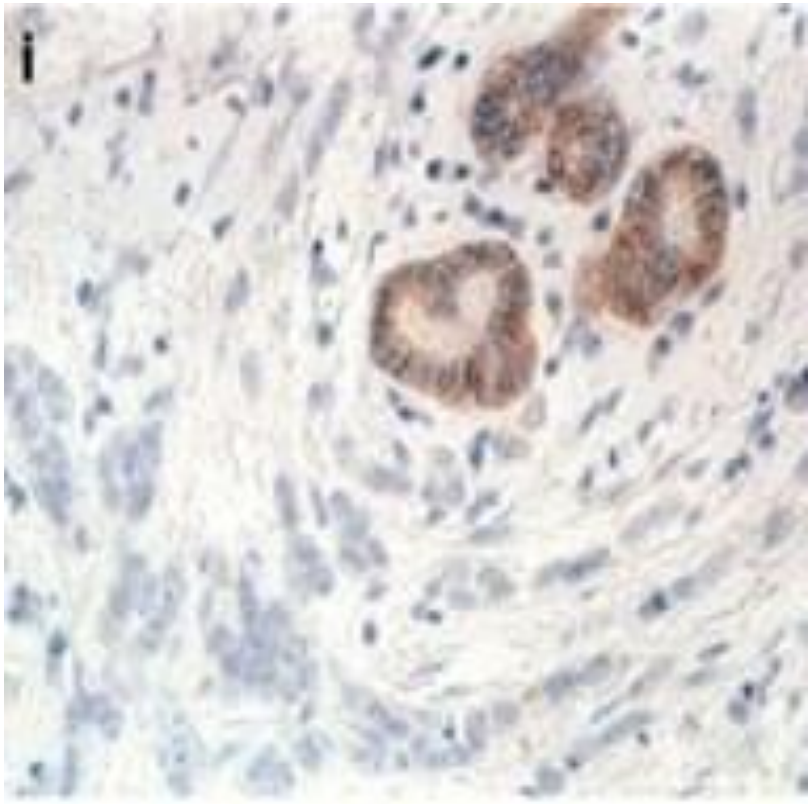
- Ductal : up to 80%
- Lobular: 5 - 14%
- Tubular: 2 - 8%
- Mucinous: 2 - 4 %
- Apocrine: 1 – 4%
- Papillary 1 – 2%
- Other


Tubular Carcinoma

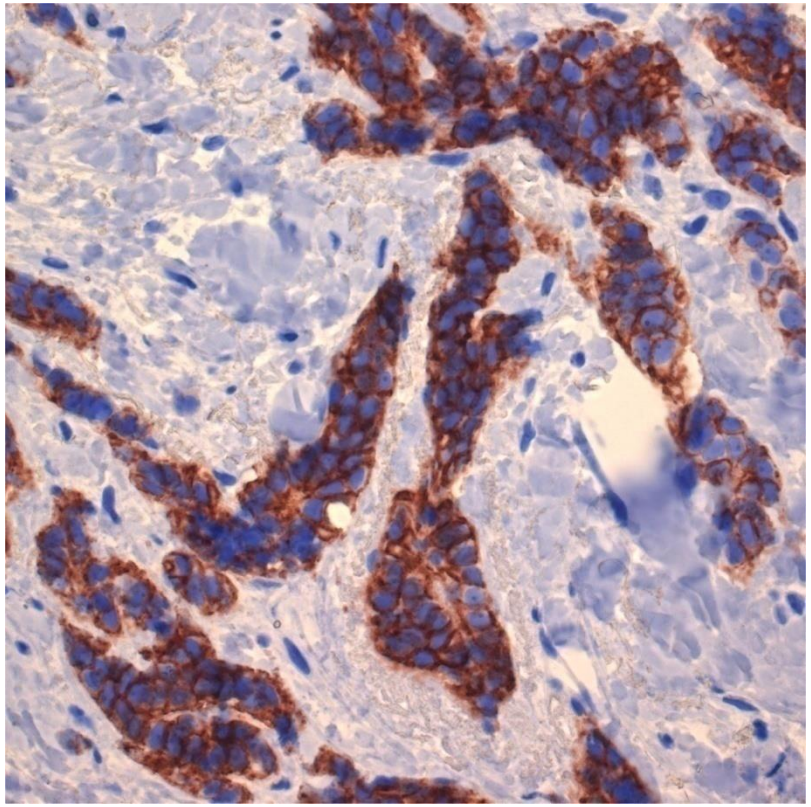

Mucinous Carcinoma

E-Cadherin
Cell adhesion molecule

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

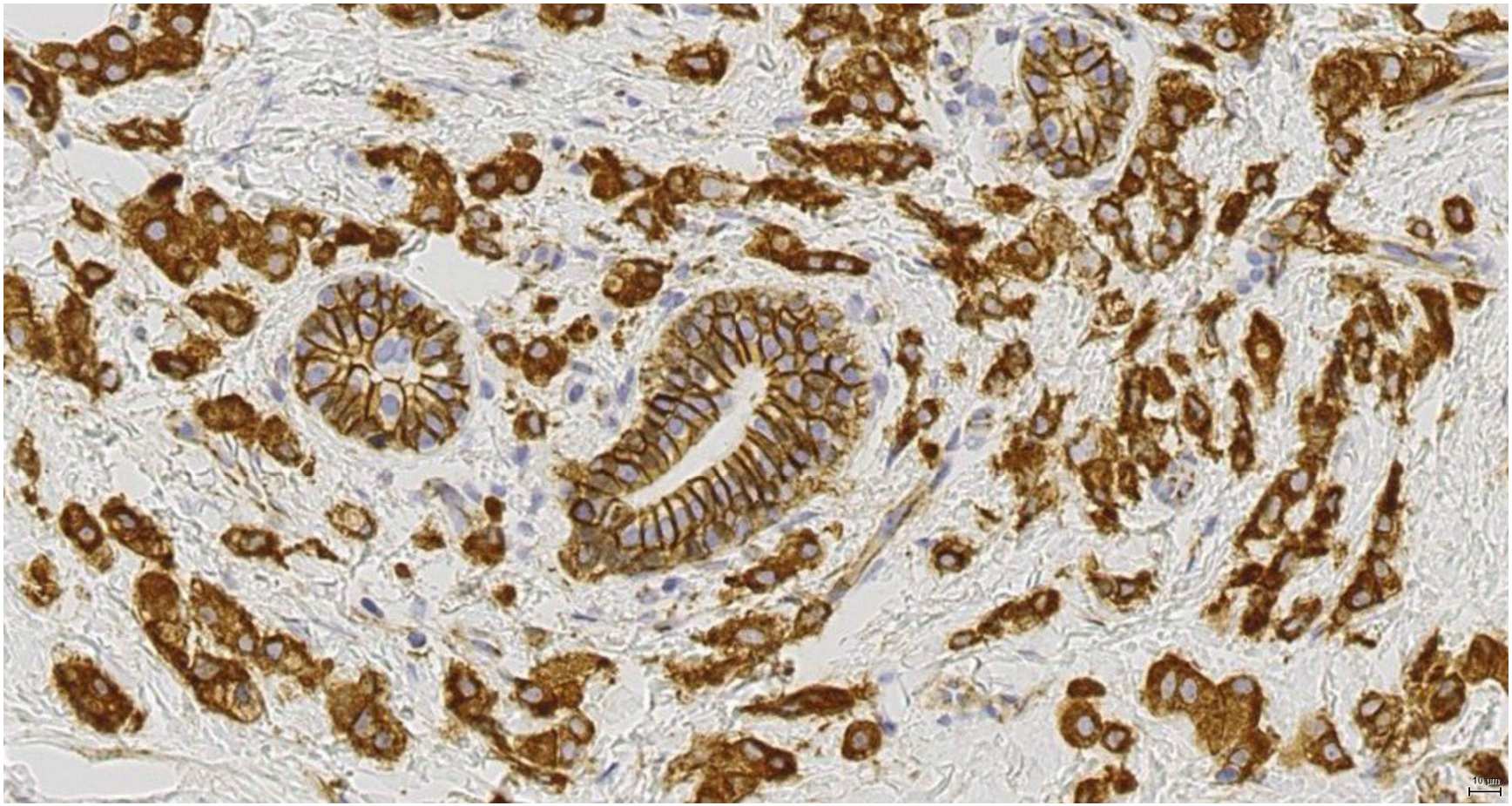


E-Cadherin positive Invasive Ductal Carcinoma



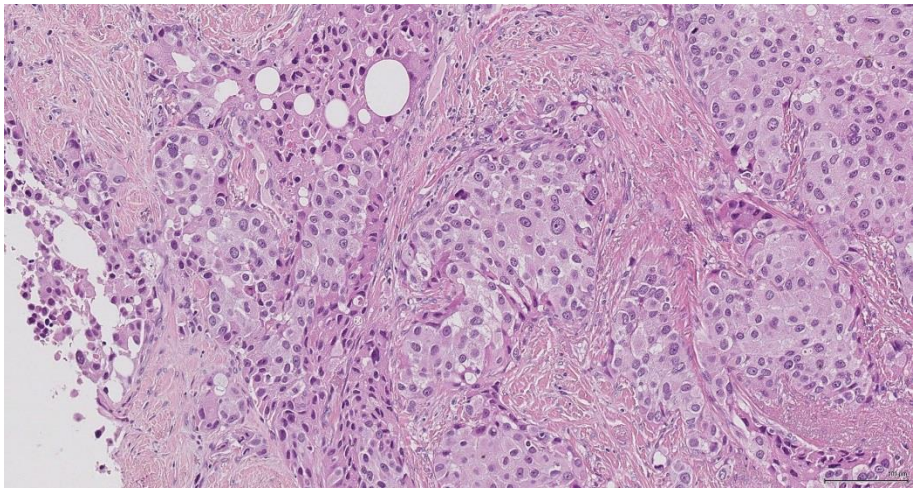
Lobular carcinoma not recommended for neoadjuvant treatment

P120 catenin dislocated to the cytoplam in lobular carcinoma

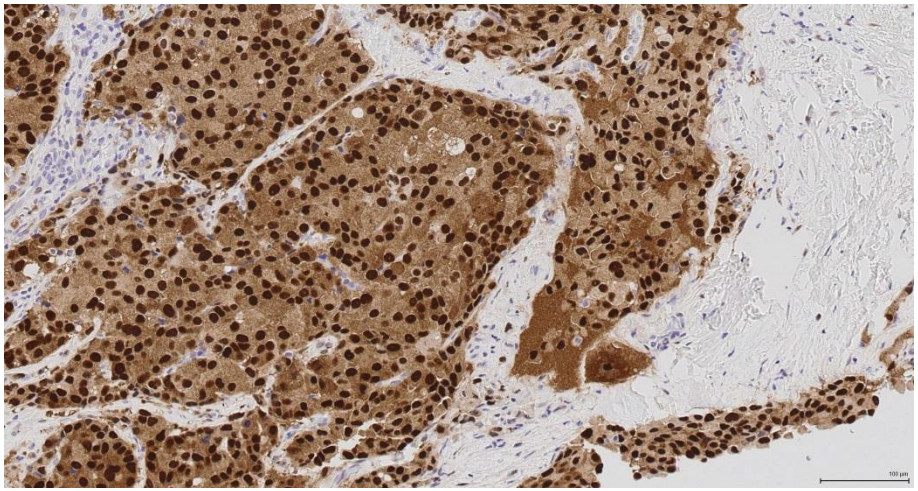


Apocrine carcinoma classification

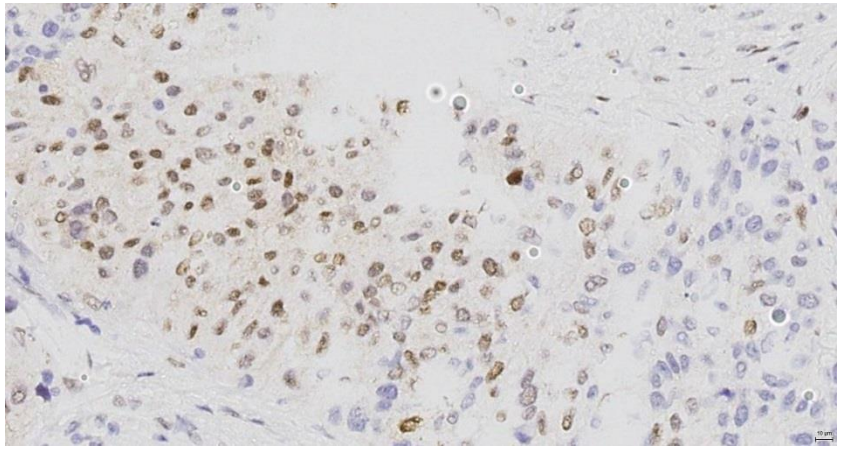
HE



Androgen Receptor

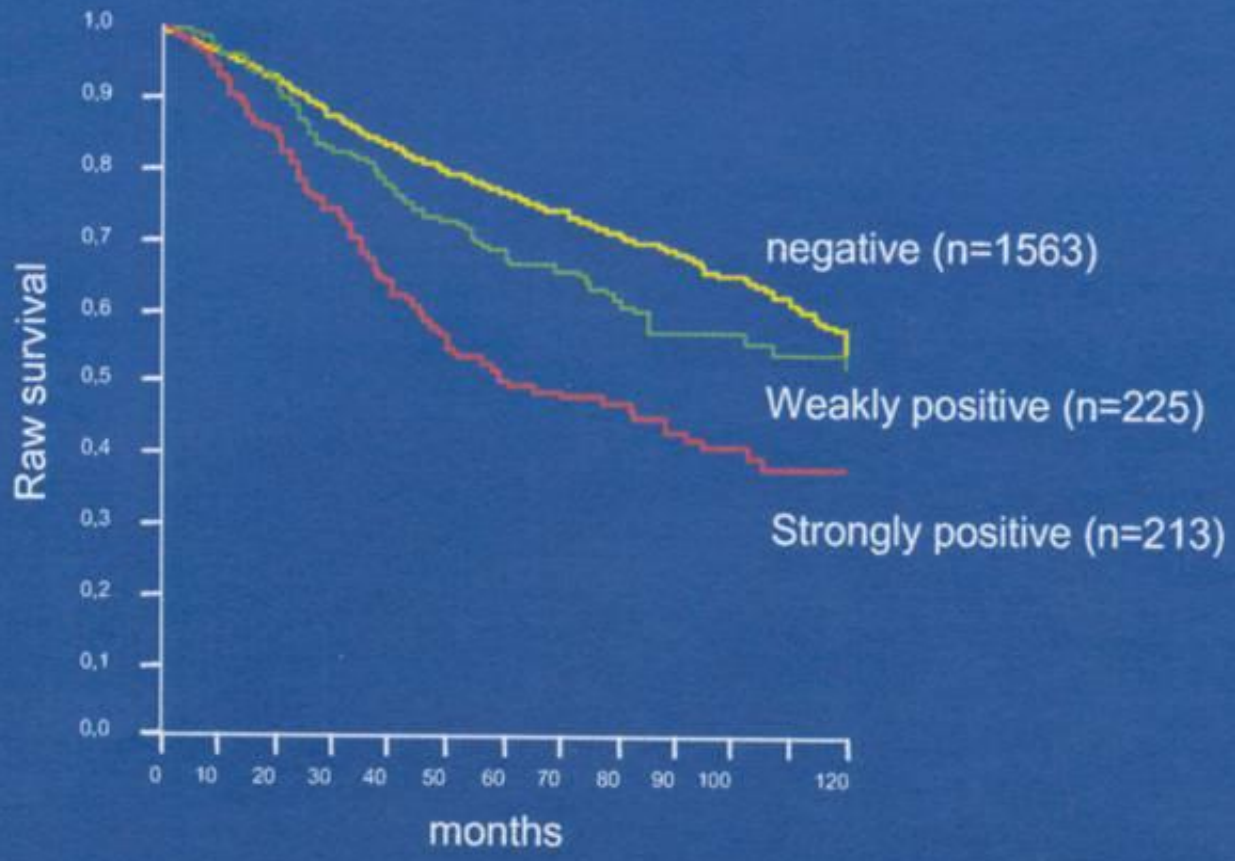


AR staining in IHC-basallike breast cancer as potential marker for AR targeted treatment



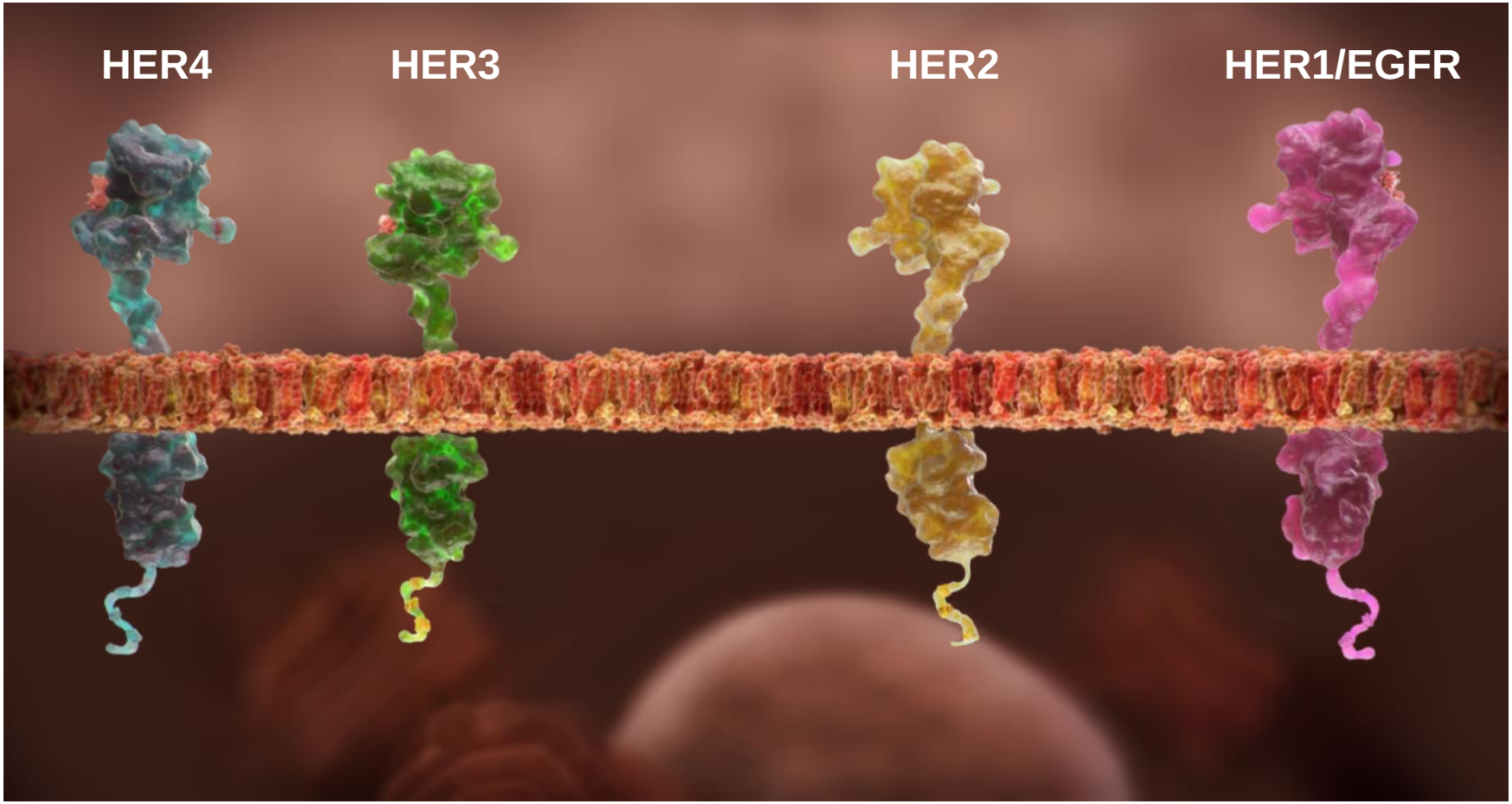
Prognostic and predictive biomarkers

HER2 and Breast Cancer Progression



Science, Vol 235, 1987

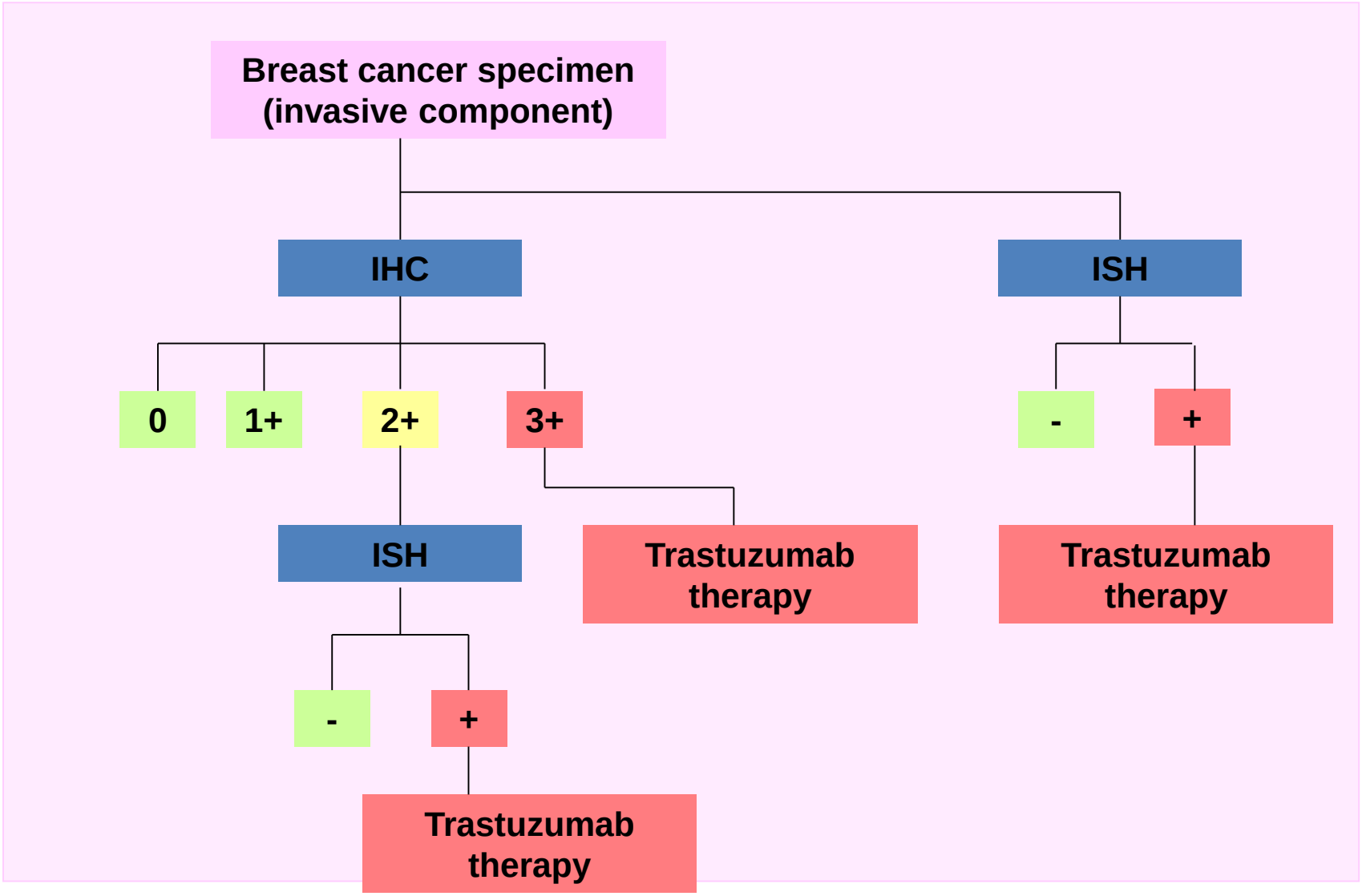
There are four receptors in the HER family



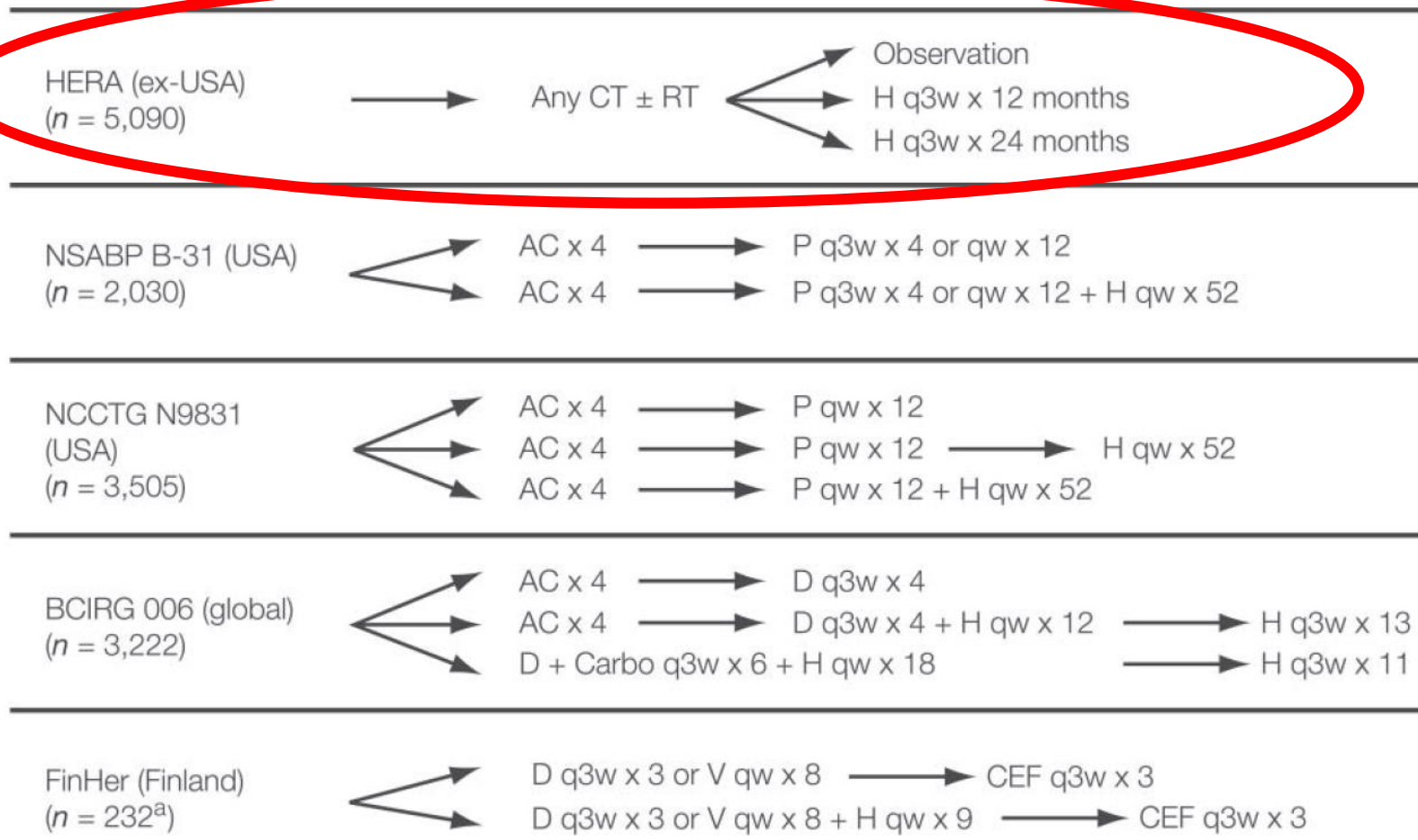
EGFR, epidermal growth factor receptor; HER, human epidermal growth factor

Adapted from
Yarden Y & Sliwkowski MX. *Nat Rev Mol Cell Biol* 2001; **2**:127–137.

HER2 Algoritm



HER2 trials for early breast cancer 2000-2001



HERA; 11 years follow up – final analysis

- After 11 years of median follow-up, the use of 1 year of adjuvant trastuzumab significantly improves disease outcomes in patients with HER2-positive early breast cancer.
- The relative risk of a disease-free survival event is reduced by 24%.
- An absolute benefit of 6.8% improvement in 10-year disease-free survival in those women who were randomly assigned to 1-year trastuzumab group compared with those assigned to the observation group.
- A 6.5% absolute gain was found in overall survival at 12 years between those in the 1-year trastuzumab group versus those in the observation group.

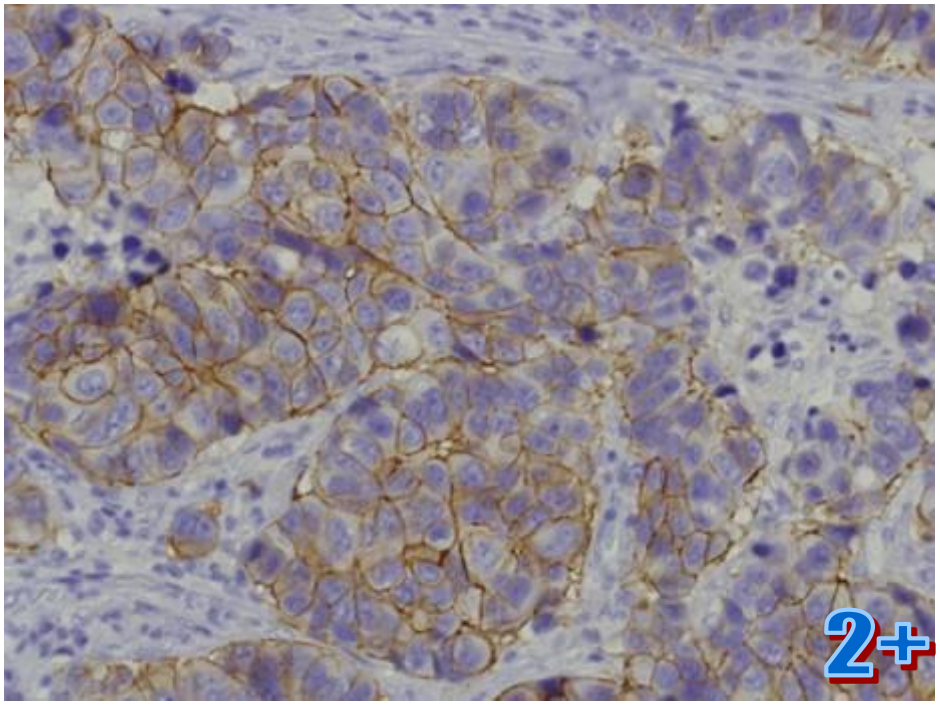
Lancet. 2017 March 25; 389(10075): 1195–1205.

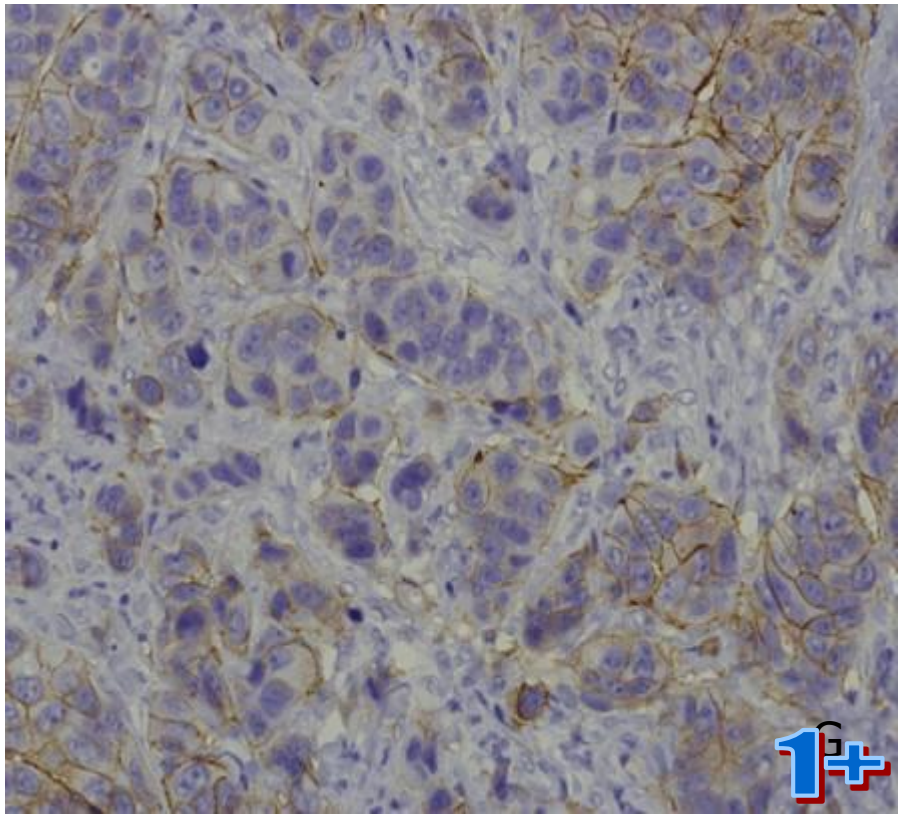
Two different assays

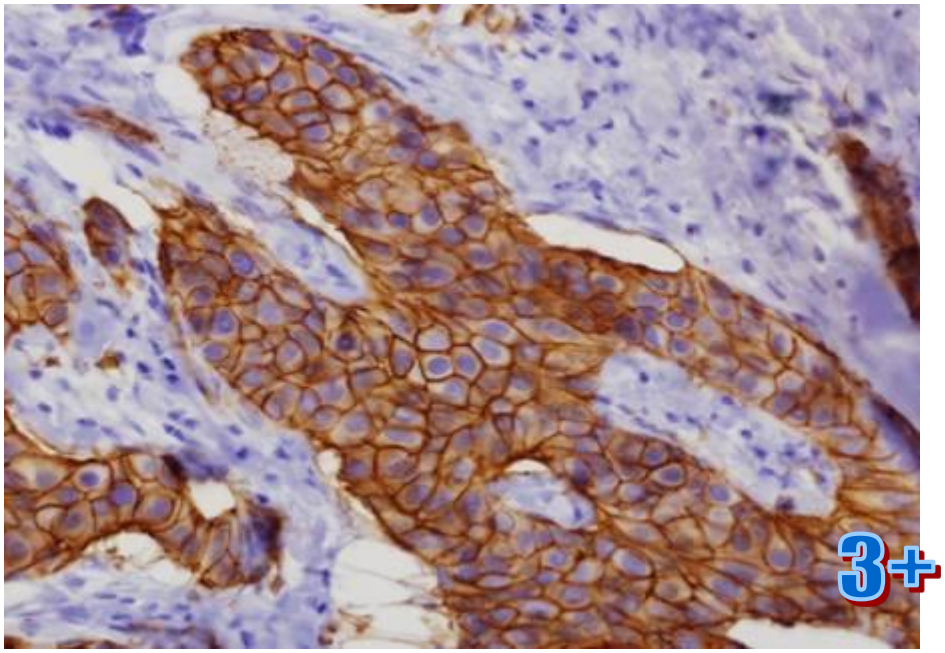
- IHC is an assay at the single-cell level
  - It will detect even an individual positive cell
- ISH is a population-based assay (mean number of gene copies/cell evaluated by scoring 20-60 cells.)
  - The final result depends on the number of gene copies of the amplified cells after dilution by non-amplified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

HER2 IHC

Obs invasive micropapillary carcinoma

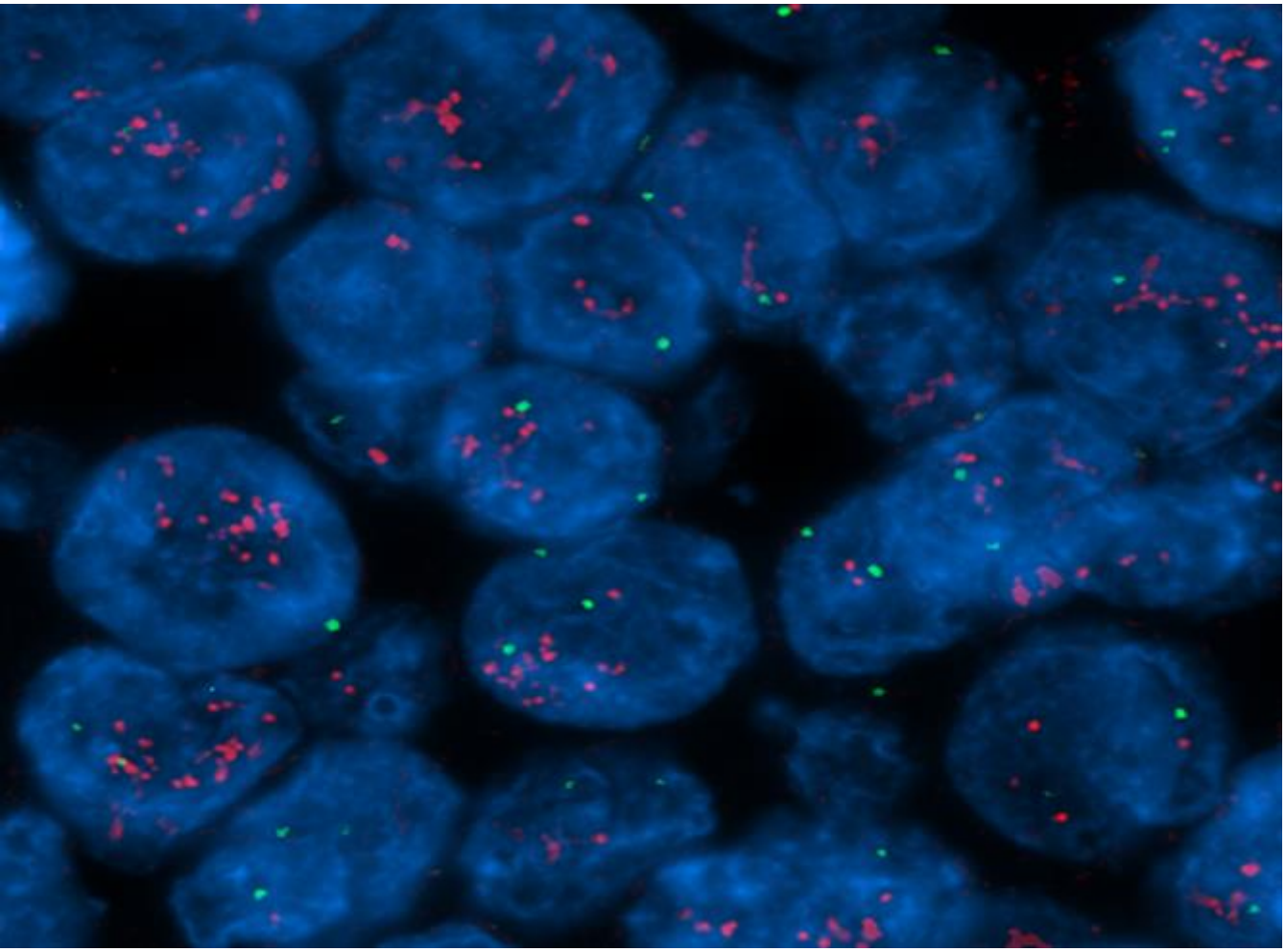






HER2 3+ and ISH + : 15 % (DK)

HER2 FISH



Green: :centromere chromosome 17

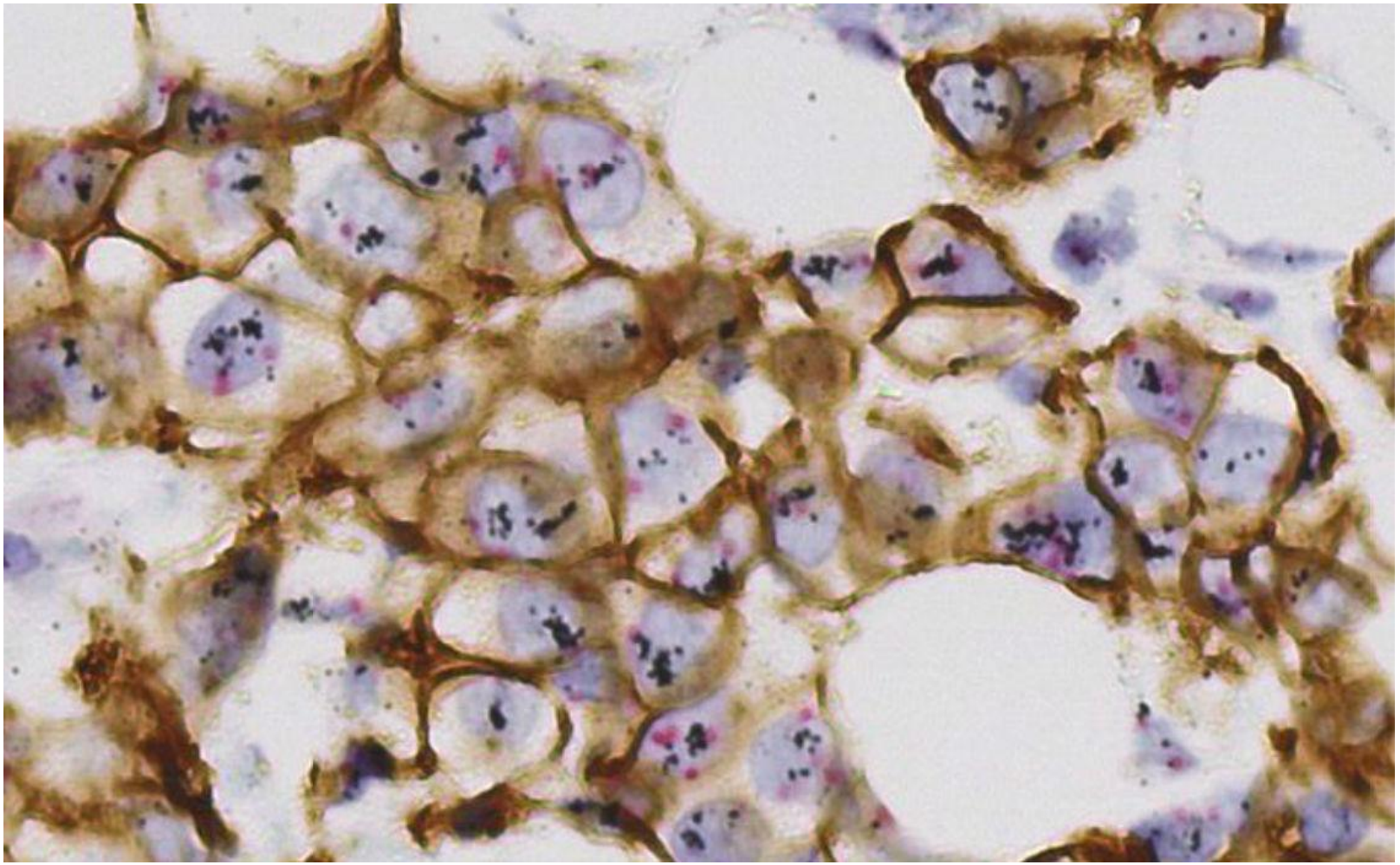
Red : HER2 gene

Dual probe:

Amplified

HER2/CEN17 ratio > 2.0

HER2 Gene/Protein Assay



HER2 amplified and HER2 IHC 3+

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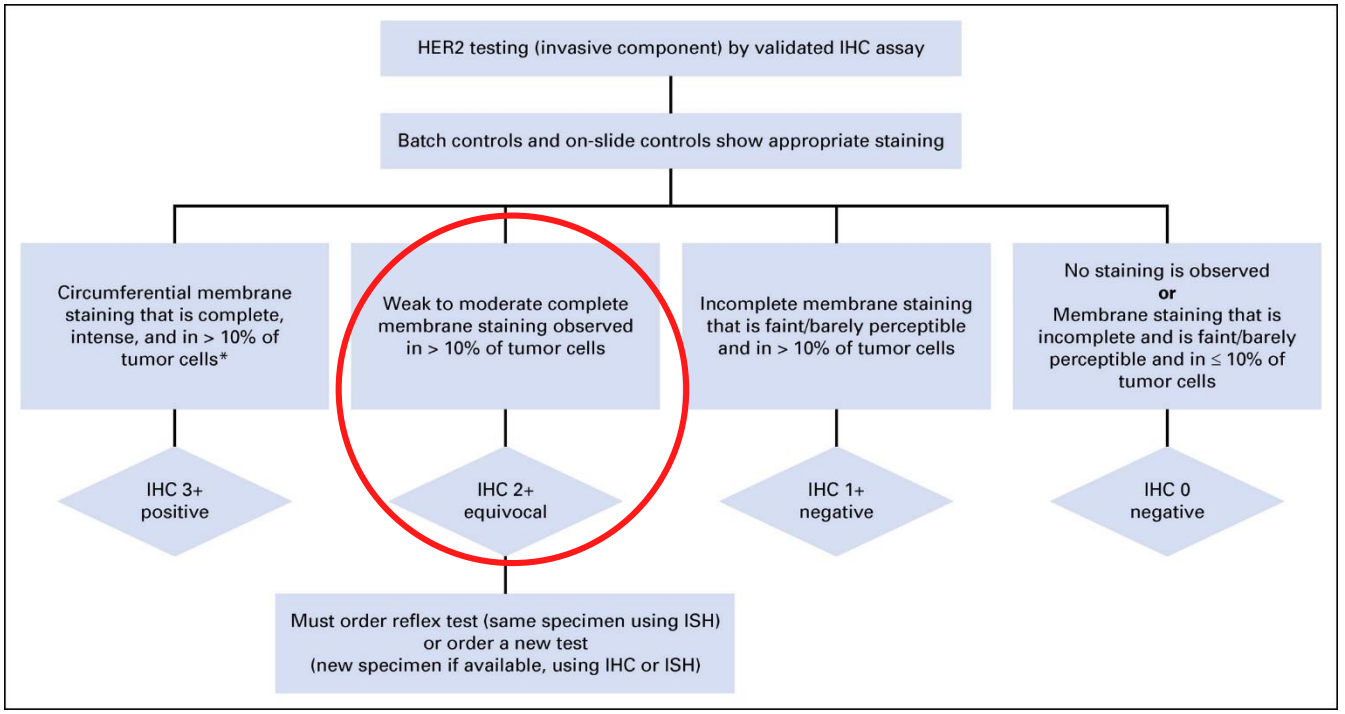
JOURNAL OF CLINICAL ONCOLOGY

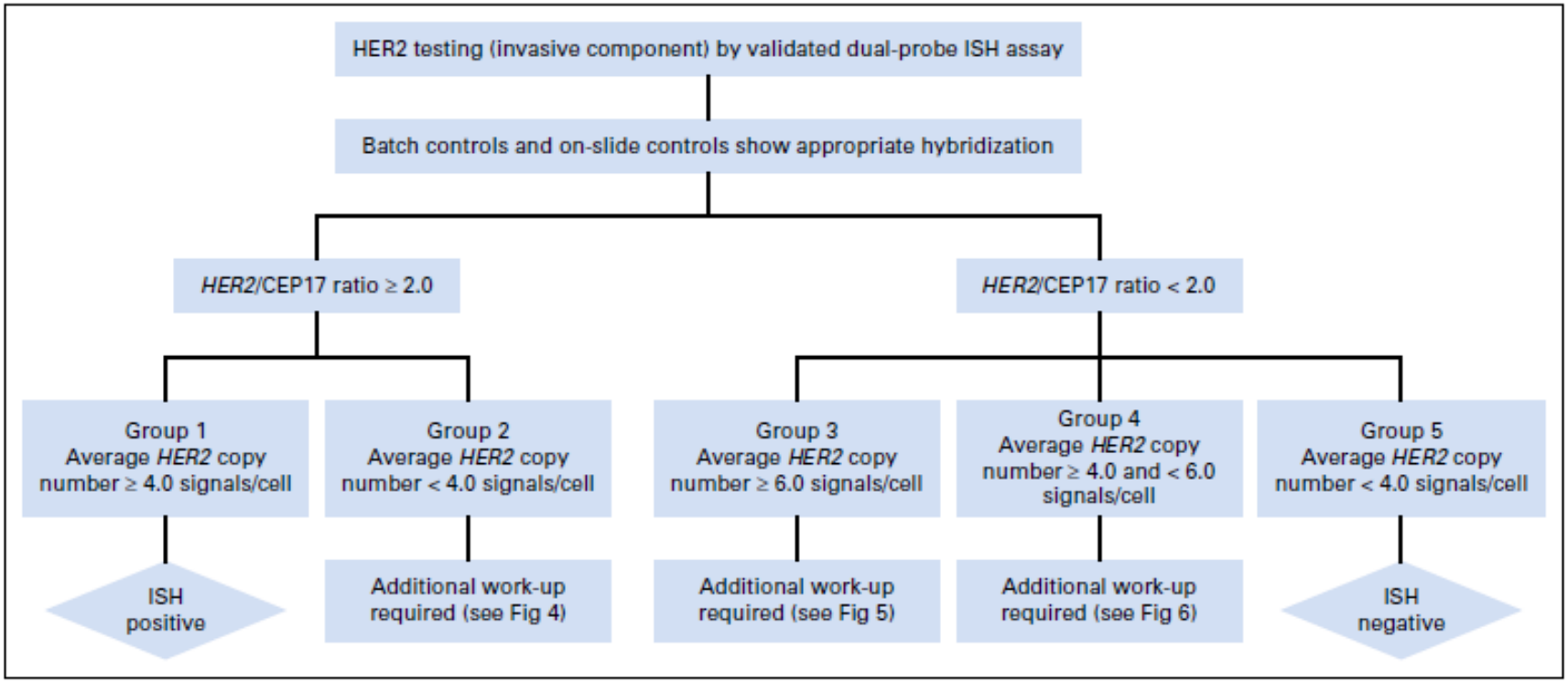
ASCO SPECIAL ARTICLE

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

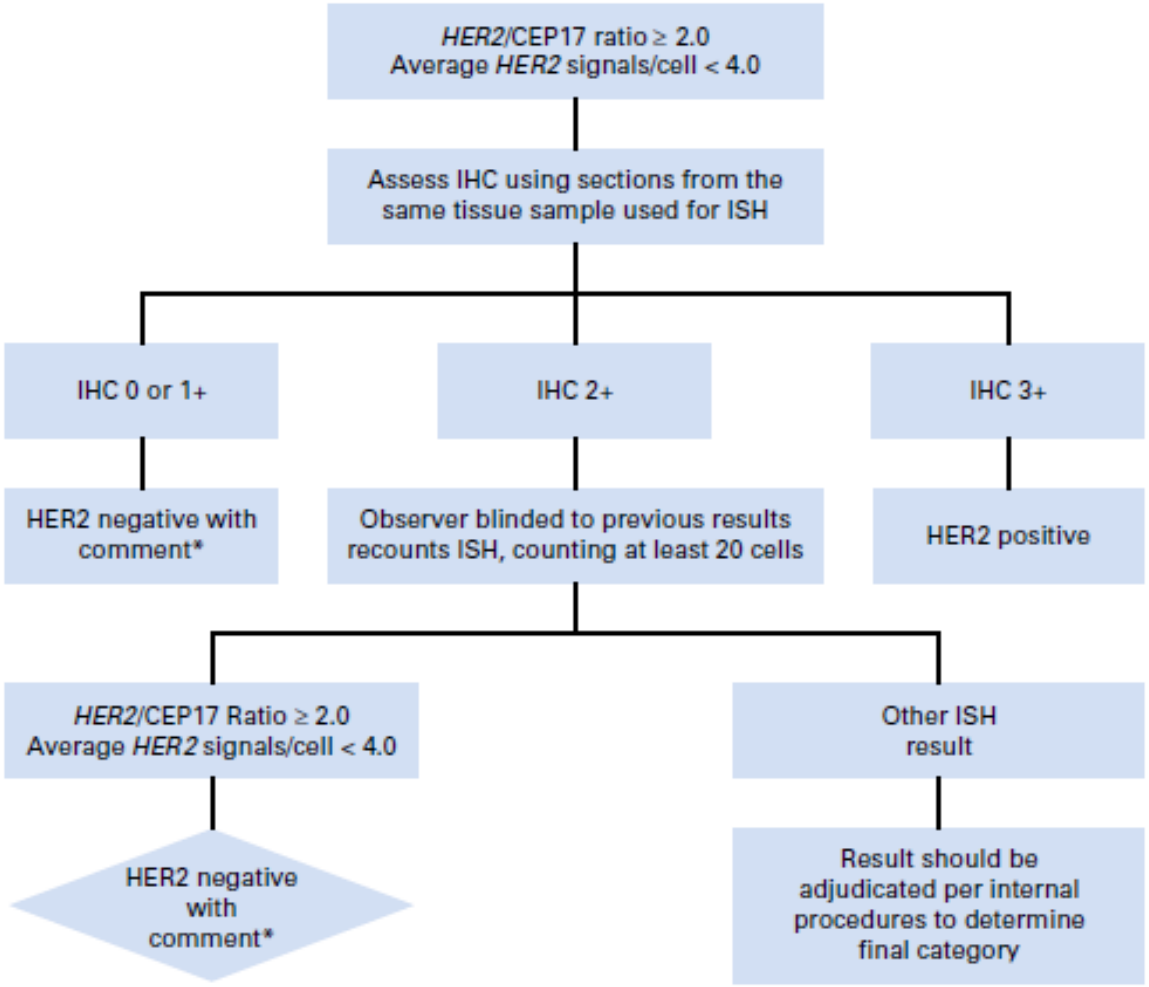
Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, and Mitchell Dowsett



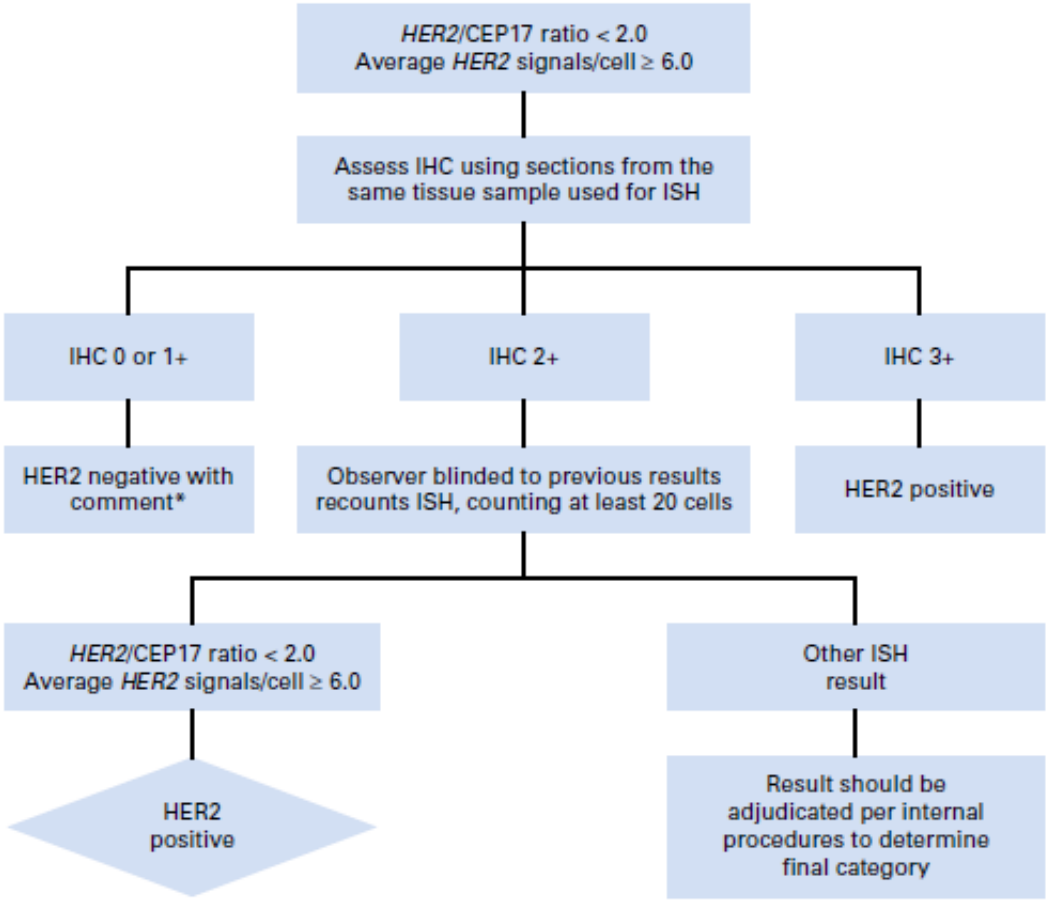
HER2 testing by validated dual-probe ISH assay



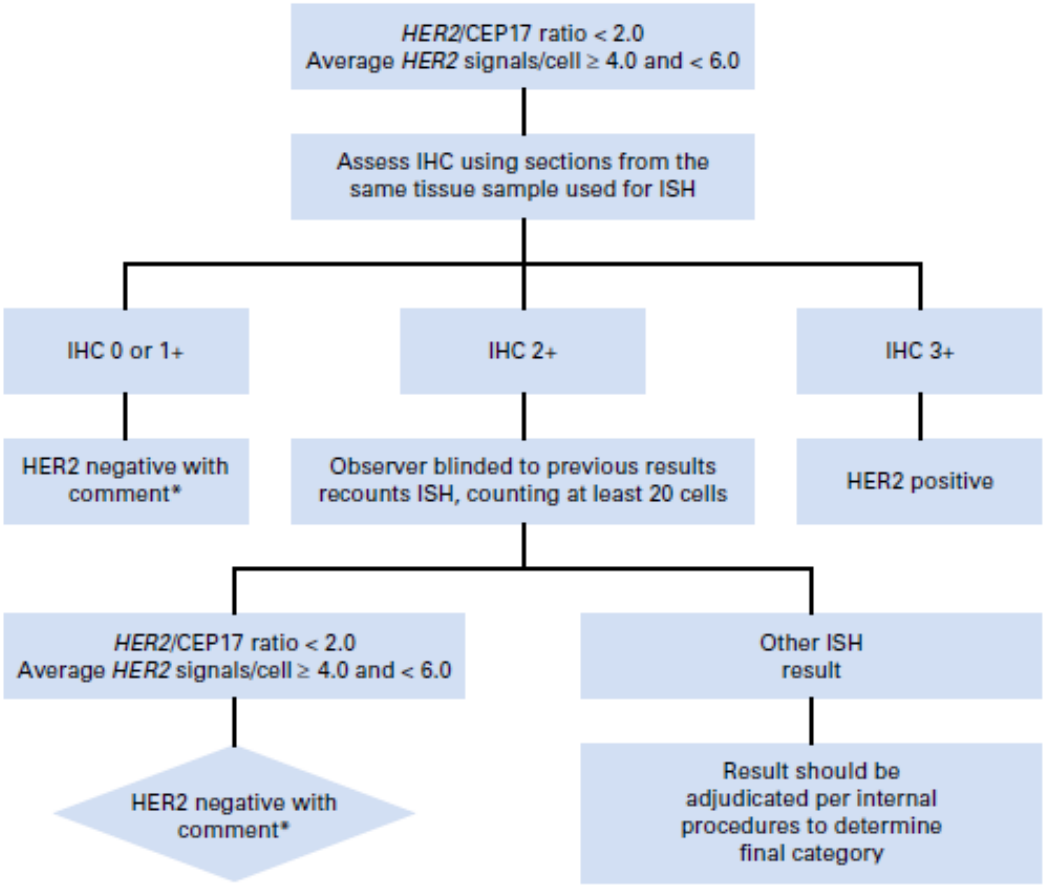
Group 2


HER2/CEP17 ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 Ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

Group 3

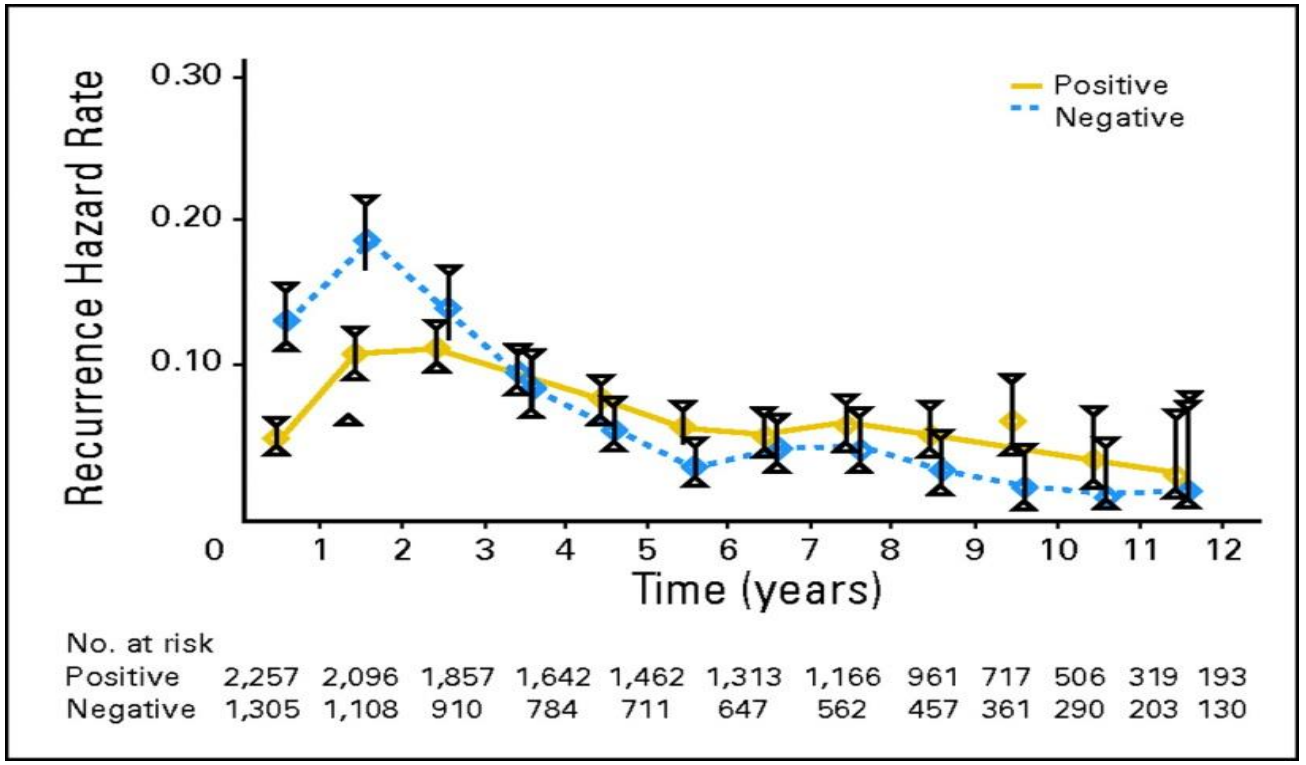

HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Other ISH result
HER2 positive
Result should be adjudicated per internal procedures to determine final category

Group 4


HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 4.0 and < 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 4.0 and < 6.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

The oestrogen receptor as a prognostic marker
Shift from prognostic to predictive!!

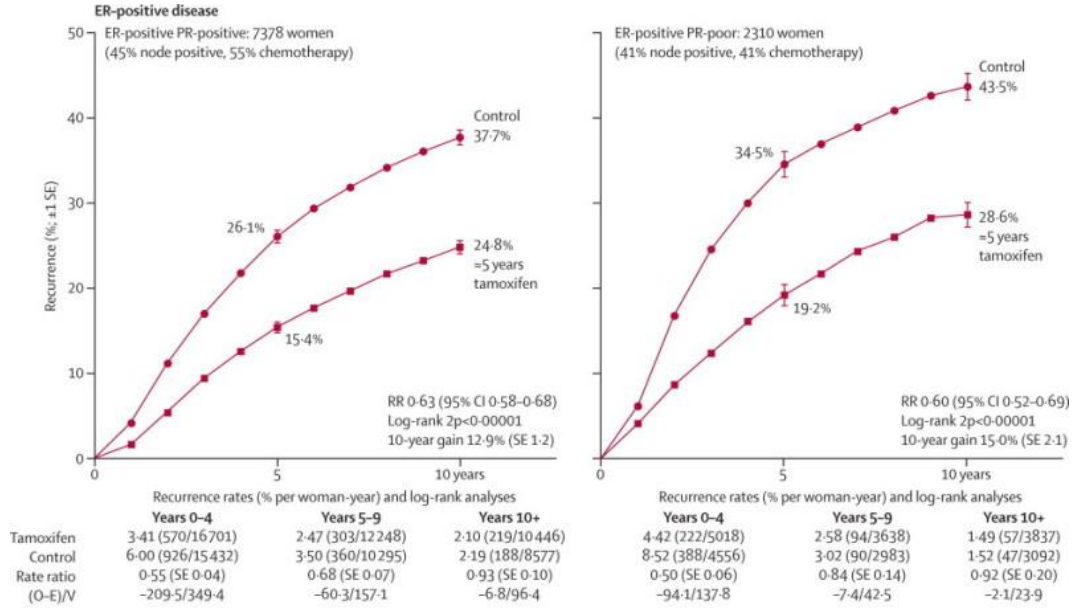
Risk of recurrence pr. year
N = 3,562 patients



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

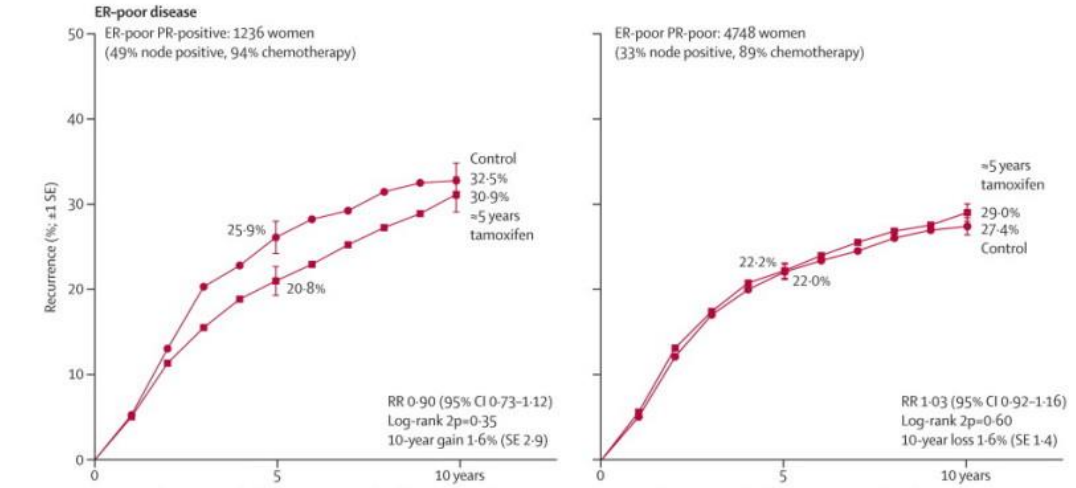
Relevance of measured ER and PR status on the effects of about 5 years of tamoxifen on the 10 year probability of recurrence (EBCTCG)

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



	Years 0–4	Years 5–9	Years 10+
Tamoxifen	3·41 (570/16701)	2·47 (303/12248)	2·10 (219/10446)
Control	6·00 (926/15432)	3·50 (360/10295)	2·19 (188/8577)
Rate ratio	0·55 (SE 0·04)	0·68 (SE 0·07)	0·93 (SE 0·10)
(O–E)/V	−209·5/349·4	−60·3/157·1	−6·8/96·4

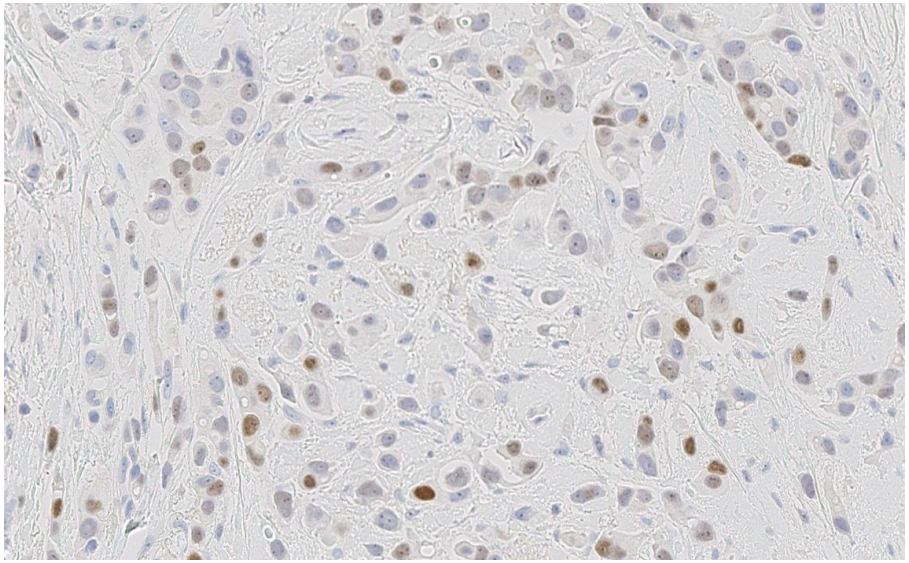
	Years 0–4	Years 5–9	Years 10+
Tamoxifen	4·42 (222/5018)	2·58 (94/3638)	1·49 (57/3837)
Control	8·52 (388/4556)	3·02 (90/2983)	1·52 (47/3092)
Rate ratio	0·50 (SE 0·06)	0·84 (SE 0·14)	0·92 (SE 0·20)
(O–E)/V	−94·1/137·8	−7·4/42·5	−2·1/23·9

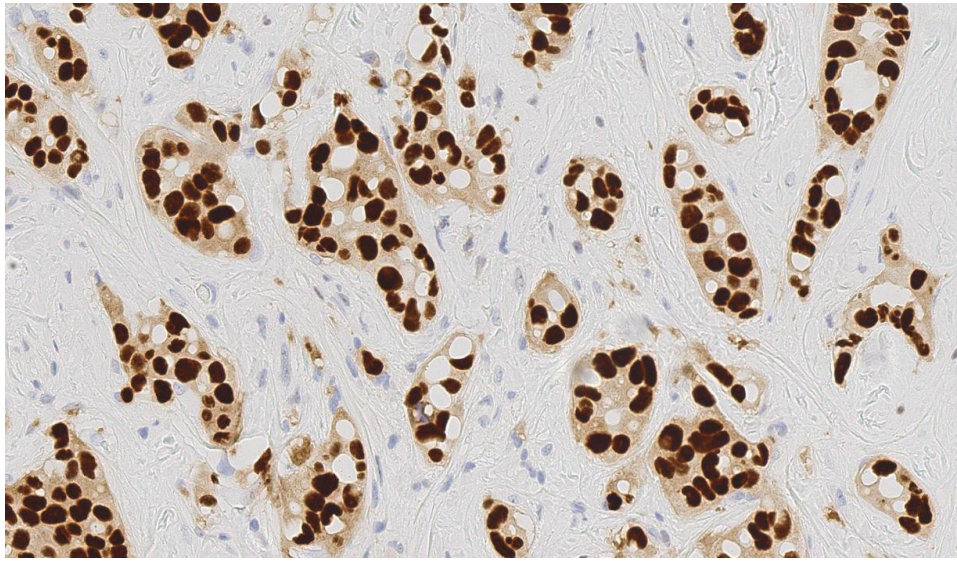


	Years 0–4	Years 5–9	Year 10+
Tamoxifen	4·66 (122/2616)	2·74 (46/1677)	1·88 (12/640)
Control	6·23 (158/2538)	1·93 (31/1603)	1·04 (7/675)
Rate ratio	0·78 (SE 0·11)	1·27 (SE 0·28)	2·03 (SE 0·69)

	Years 0–4	Years 5–9	Years 10+
Tamoxifen	5·26 (519/9870)	1·86 (113/6081)	1·09 (29/2652)
Control	5·05 (493/9754)	1·50 (93/6183)	1·45 (43/2961)
Rate ratio	1·02 (SE 0·07)	1·27 (SE 0·16)	0·70 (SE 0·20)

Interpretation of ER IHC

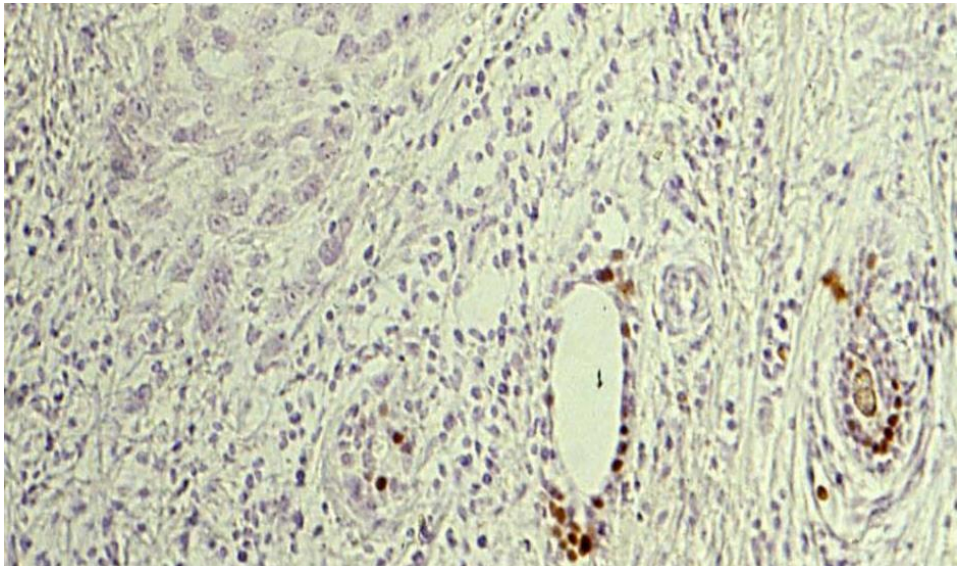




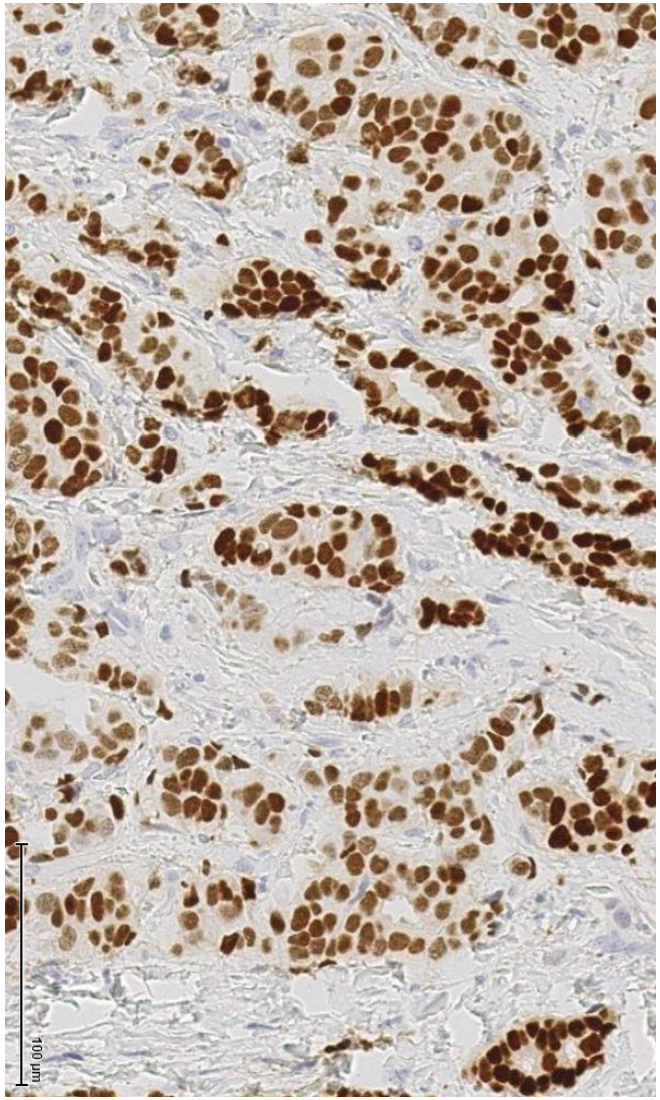
ER positive 86% of breast carcinomas (DK)
Cut off ≥ 1% (regardless of intensity)

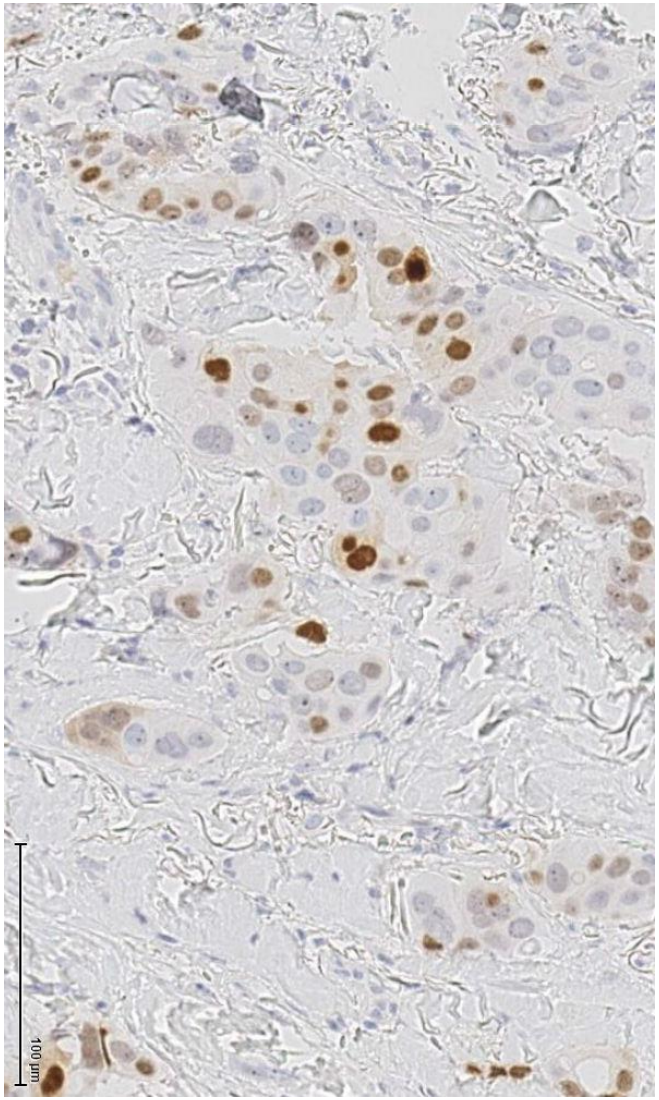
Allred method

Proportion Score (PS)	Observation	Intensity Score (IS)	Observation
0	NONE	0	None
1	1%	1	Weak
2	1-10%	2	Intermediate
3	10-33%	3	Strong
4	33-66%		
5	66-100%		
Total Score			Interpretation
Sum of proportion score and intensity score			
0-2			Negative
3-8			Positive



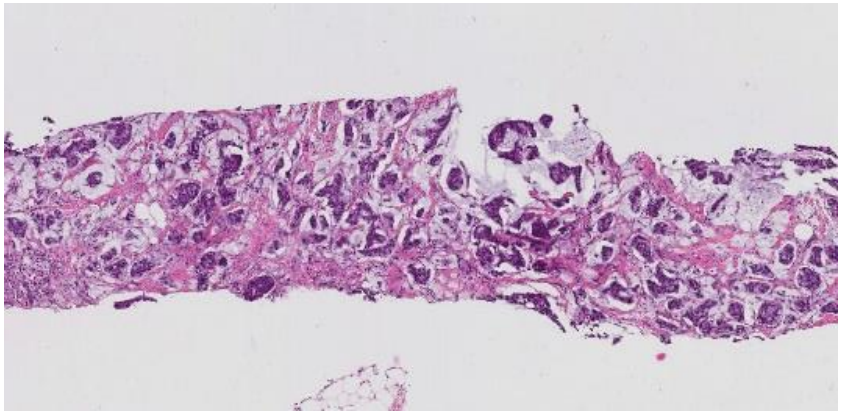
Interpretation of PgR IHC



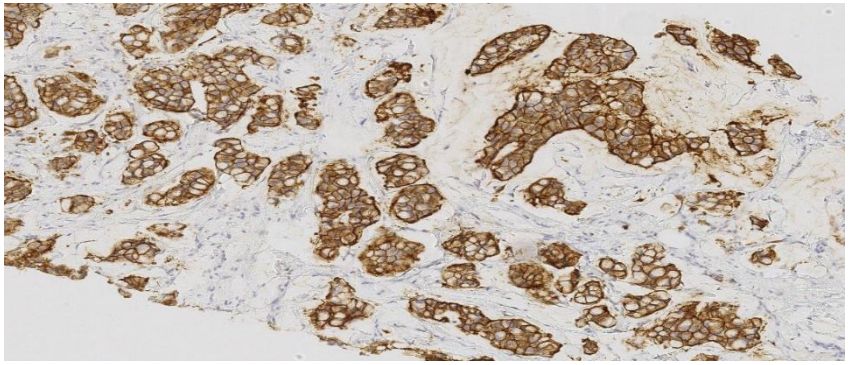


Neo-adjuvant treatment

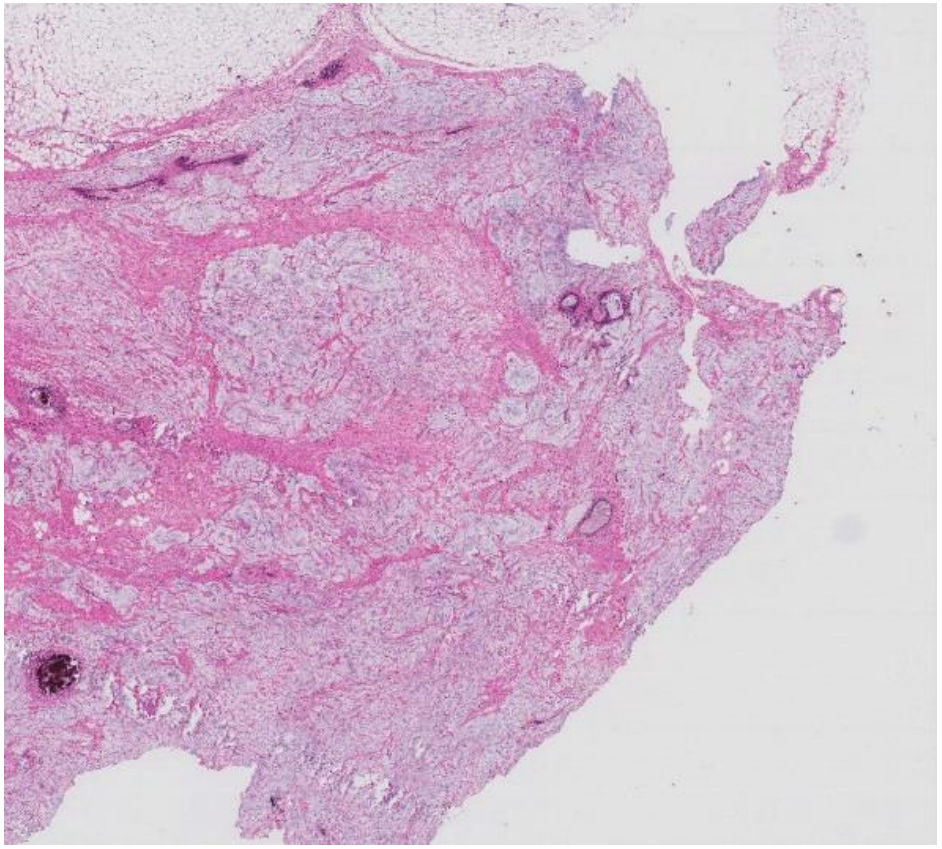
- Neoadjuvant systemic therapy in the treatment of early-stage breast cancer.
  - Tumor down staging
  - pCR (pathological complete response) is an evaluable end point for determining the efficacy of the treatment.
    - Prognostic information (DFS)



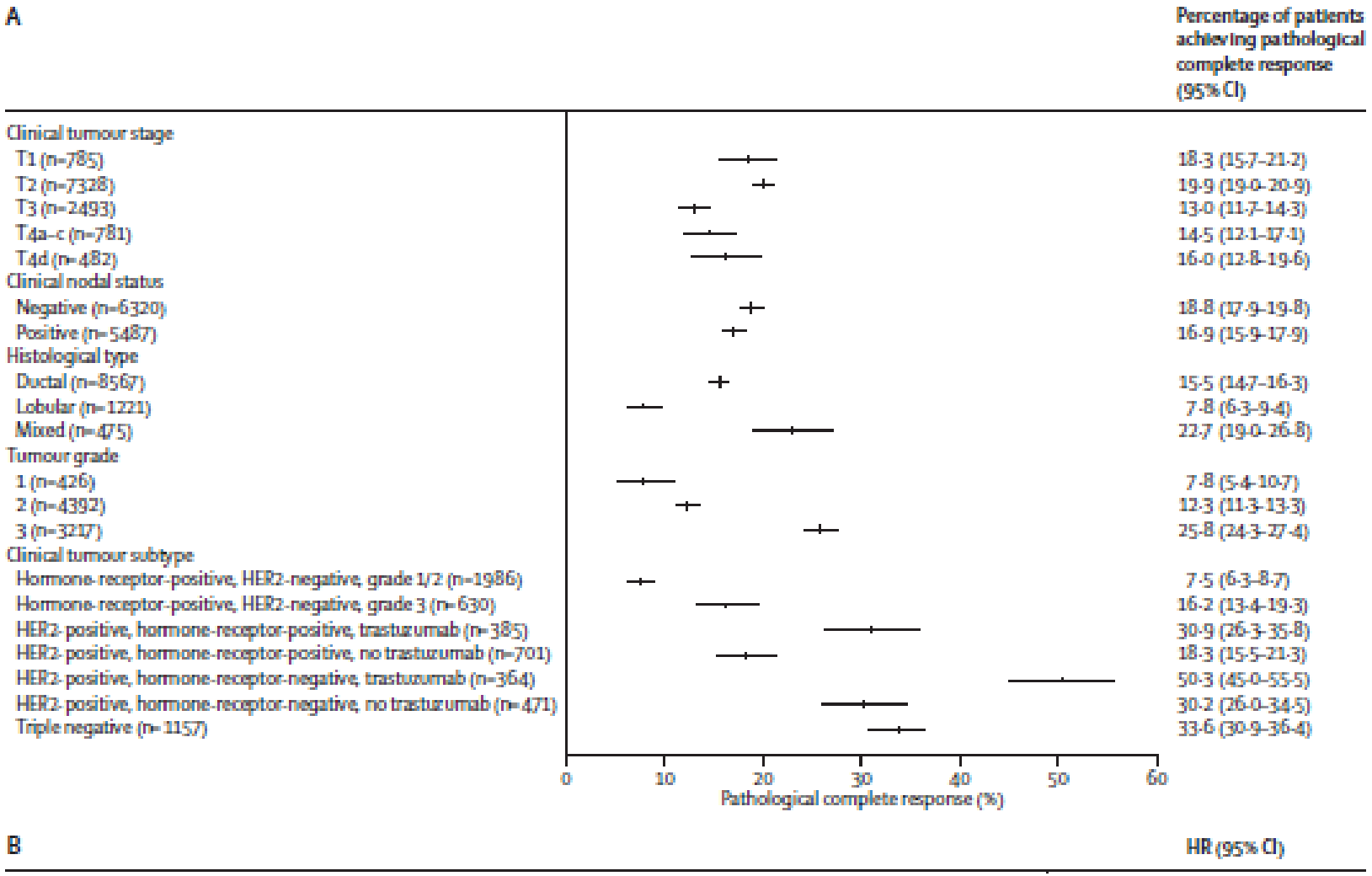
HER2 IHC



Post treatment - surgery



Tumor characteristics and association with pCR
Lobular carcinoma not recommended for neoadjuvant treatment



Cortazar et al. Lancet 2014; 384: 164-72

Neoadjuvant treatment
IHC discordancy post treatment

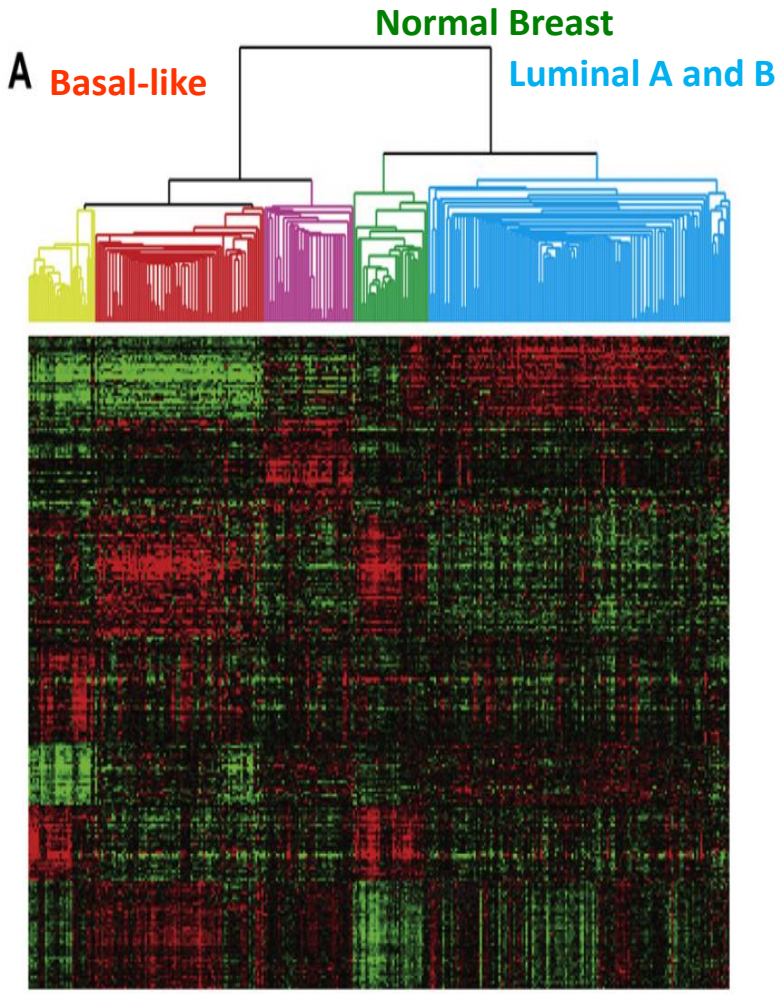
Table 2 Summary of the reported discordant ER, PR, and Her-2/neu cases post-neoadjuvant therapy

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

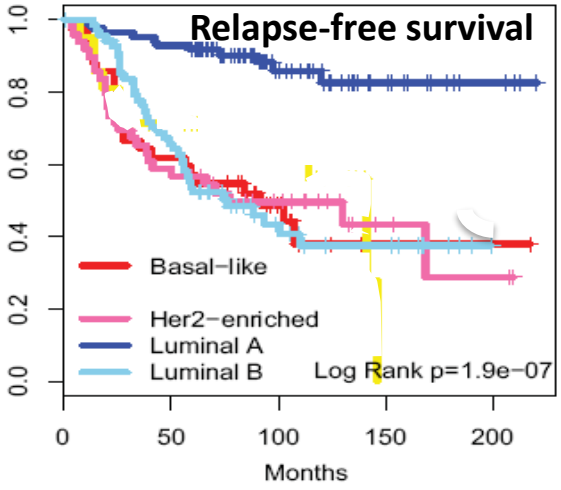
ER estrogen receptor, *PR* progesterone receptor, *Her-2/neu* epidermal growth factor receptor-2 (c-erb-2), *LR* local recurrence, *NAC* neoadjuvant chemotherapy, *IHC* immunohistochemistry, *FISH* fluorescent in situ hybridization, *DCA* dextran–charcoal assay, *HT* hormone therapy, *CT* chemotherapy

Breast Cancer Res Treat (2012) 135:29–37

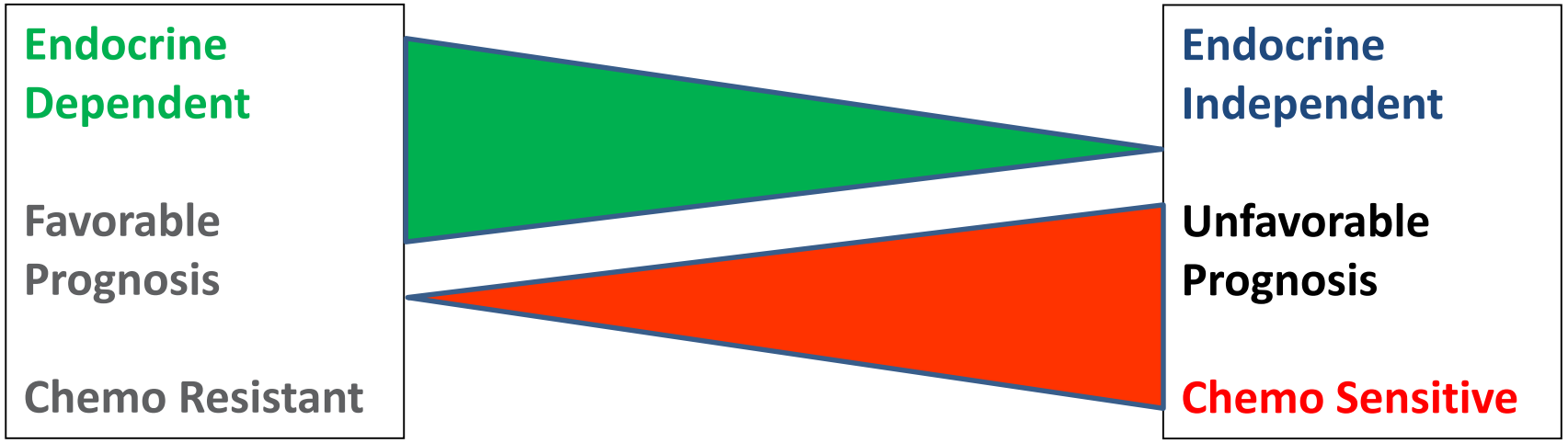
Breast cancer – Molecular intrinsic subtypes



Intrinsic Subtypes
Perou et al., Nature 2000
Sorlie et al., PNAS 2001
Sorlie et al., PNAS 2003
Nielsen et al., CCR 2004
Cheang et al., CCR 2008
Parker et al., JCO, Feb 2009
Cheang et al., JNCI 2009
Prat et al., BCR 2010
Nielsen et al., CCR 2010



Breast cancer – Molecular intrinsic subtypes



Lum A

Lum B

HER2-enriched

Basal-like

Recent Publication in the Journal of Clinical Oncology

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

PAM50 Risk of Recurrence Score Predicts 10-Year Distant Recurrence in a Comprehensive Danish Cohort of Postmenopausal Women Allocated to 5 Years of Endocrine Therapy for Hormone Receptor–Positive Early Breast Cancer

Anne-Vibeke Lænkholm, Maj-Britt Jensen, Jens Ole Eriksen, Birgitte Bruun Rasmussen, Ann S. Knoop, Wesley Buckingham, Sean Ferree, Carl Schaper, Torsten O. Nielsen, Taryn Haffner, Torben Kibøl, Maj-Lis Møller Talman, Anne Marie Bak Jylling, Tomasz Piotr Tabor, and Bent Ejlertsen

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on January 25, 2018.

Corresponding author: Anne-Vibeke Lænkholm, MD, Department of Surgical

A B S T R A C T

Purpose

The PAM50-based Prosigna risk of recurrence (ROR) score has been validated in randomized clinical trials to predict 10-year distant recurrence (DR). The value of Prosigna for predicting DR was examined in a comprehensive nationwide Danish cohort consisting of postmenopausal women with

JOURNAL OF CLINICAL ONCOLOGY EDITORIAL

Do Genomic Assays Provide the Necessary Confidence to De-escalate Adjuvant Therapy?

Ricardo L. B. Costa, *H. Lee Moffitt Cancer Center, Tampa, FL*
William J. Gradishar, *Northwestern University, Chicago, IL*

See accompanying article doi:10.1200/JCO.2017.74.6586

The phrases precision medicine and de-escalation of therapy are being used more frequently in the same sentence when it comes to describing goals of cancer therapy. For perspective, when the National Comprehensive Cancer Network (NCCN) produced its first practice guideline for breast cancer in 1996, the recommendations for adjuvant therapy of early-stage breast cancer were rather simple, reflecting the knowledge generated from clinical trials up to that time.[1] Specifically, adjuvant treatment decisions were largely based on age, estrogen receptor (ER) status, tumor size, and the number of axillary nodes involved.

The greater accumulation of clinical trial data married with a far greater understanding of cancer biology has resulted in better outcomes for patients with early-stage disease. Antiestrogen therapy remains the cornerstone of the adjuvant treatment of patients with ER-positive/human epidermal growth factor receptor 2 (HER2)–negative, early-stage breast cancer. Indeed, in a meta-analysis of randomized trials pooling data from 10,645 patients with ER-positive breast cancer, adjuvant treatment with tamoxifen for 5 years significantly reduced not only breast cancer recurrence rates for 10 years but also led to improvement in the risk breast cancer–related mortality; the relative risk was reduced by approximately 30% throughout the first 15 years from initiation of treatment.[2] Adjuvant treatment with chemotherapy can also further reduce the probability of breast recurrence in a subset of patients with localized disease. Results of meta-analyses also conducted under the auspices of the Early Breast Cancer Trialists' Collaborative Group showed that, among 8,575 women, adjuvant treatment with an anthracyclines-based regimen correlated with a relative risk of breast cancer–related mortality of 0.79 when compared with no

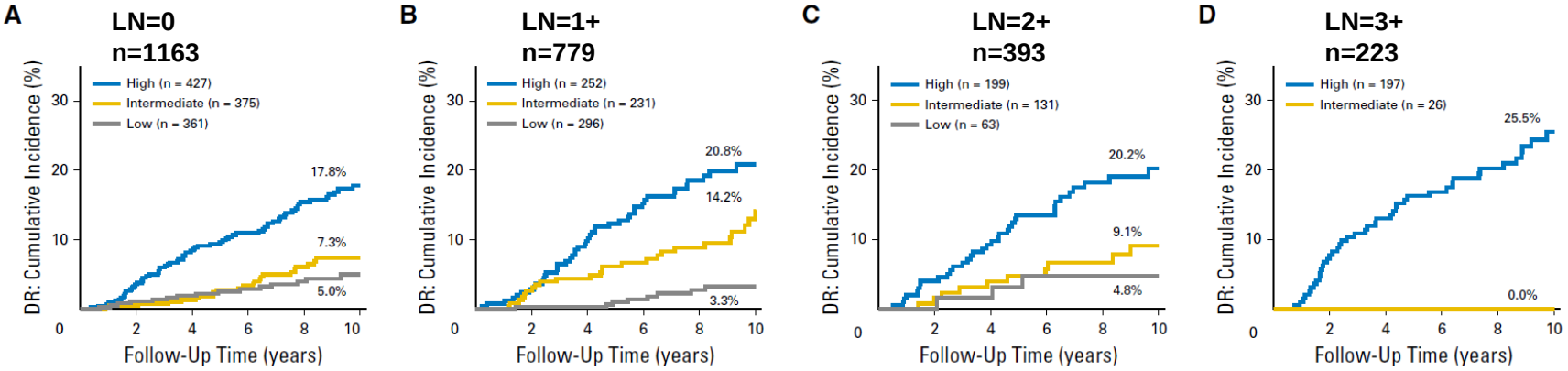
provided prognostic information and, more importantly, was able to categorize groups of patients with ER-positive, node-negative breast cancer who had such a good prognosis at 10 years with endocrine therapy alone that chemotherapy would not provide additional benefit (predictive).[4] The analysis also identified a group at high risk for recurrence at 10 years in whom the added benefit of chemotherapy was clear. There is also an intermediate group in whom the added benefit of chemotherapy was less clear, and it is that subset of patients that is now subject of a large clinical trial (TAILOR-X) to better define the contribution of chemotherapy. The use of this assay has been endorsed by NCCN and ASCO guidelines for over a decade to aid clinical decision-making. The added value of this assay can also be viewed through the lens of clinician recommendations that were changed to, or against, chemotherapy on the basis of results of the assay in patients with node-negative breast cancer.

With an appreciation that it is not clinical features alone but rather the partnering of clinical and molecular features that are the codrivers of any given tumor, the importance of biology has become a key focus in clinical decision-making. For instance, it has long been appreciated that not all node-positive breast cancers will recur even in the absence of any systemic adjuvant therapy. Additionally, even in the era of systemic adjuvant therapy, there are patients with early-stage, ER-positive, node-positive cancer who receive endocrine therapy and in whom disease does not recur in the absence of chemotherapy. Believing that it is more than happenstance and, likely, biology that drives these tumors toward a more favorable clinical course, investigators have explored whether molecular assays may identify those patients with ER-positive,

Major findings from this study – with regards to distant recurrence risk at 10 years after 5 years of endocrine therapy alone

		1395)
Low risk		**6.1%)**
Intermedia		**15.6%)**
High risk		**o 25.8%)**

All patients categorized as low risk regardless of nodal involvement status had DR absolute risk below or equal to 5%



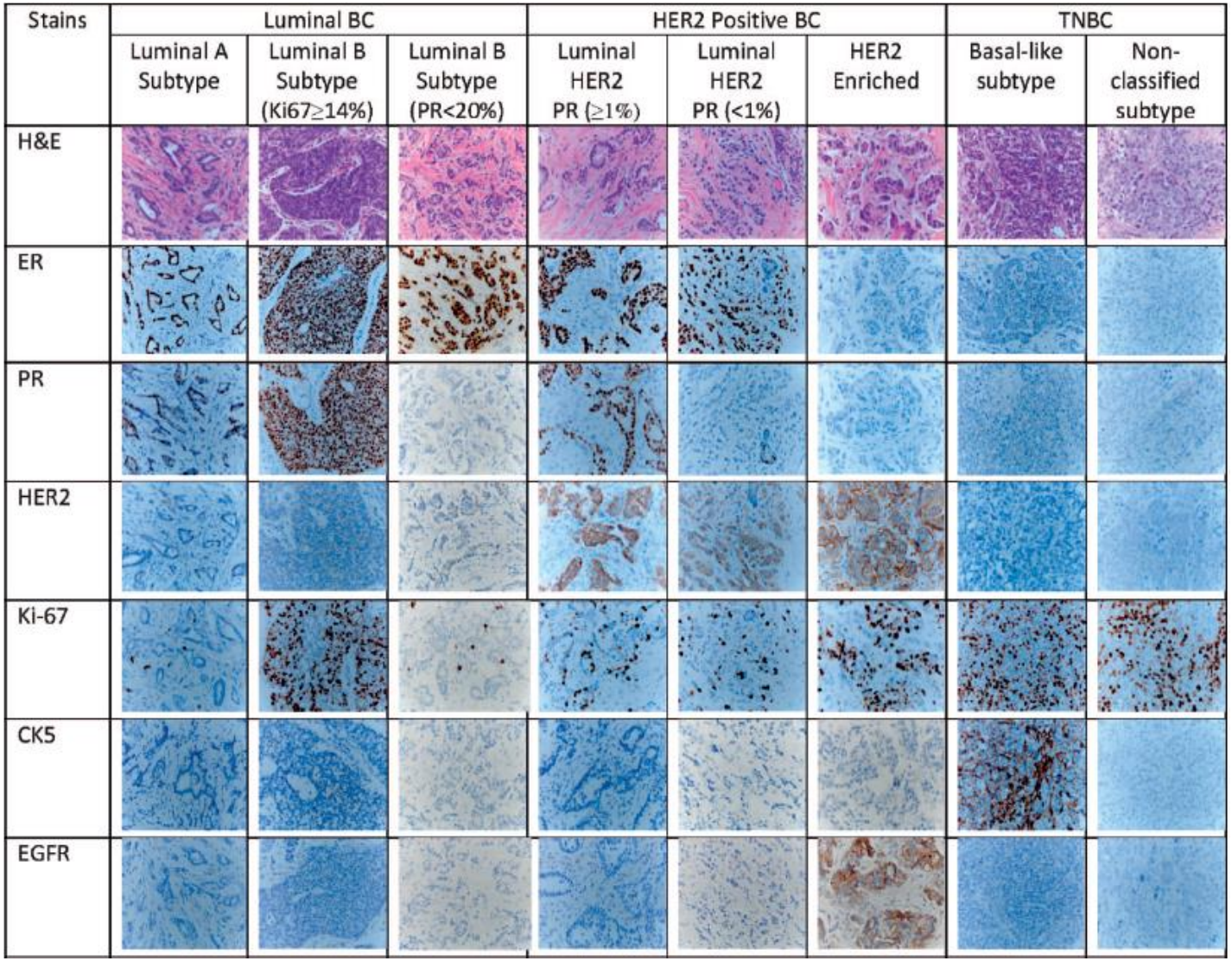
De-escalation of treatment
More patients can be spared chemotherapy

Immunohistochemical surrogate markers for the molecular intrinsic subtypes

- Limitations
  - Confusing terminology i.e.
    - basallike breast cancer vs triple negative breast cancer
  - No uniform cut off
  - Lack of correlation: molecular subtypes and surrogate IHC subtypes

Immunohistochemical surrogate markers for the molecular intrinsic subtypes

Arch Pathol Lab Med—Vol 140, August 2016



Development of an improved panel for basal breast cancer

A survey of immunohistochemical biomarkers for basal-like breast cancer against a gene expression profile gold standard

Jennifer R Won[1,2], Dongxia Gao[2], Christine Chow[2], Jinjin Cheng[2], Sherman YH Lau[2], Matthew J Ellis[3], Charles M Perou[4], Philip S Bernard[5] and Torsten O Nielsen[1,2]

[1]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; [2]Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, British Columbia, Canada; [3]Division of Oncology, Department of Internal Medicine, Washington University, St Louis, MO, USA; [4]Department of Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, USA and [5]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT, USA

- **46** proposed IHC biomarkers published in the literature as associated with the basal subtype
- Utilizing PAM50 gene expression profiling platform as a gold standard

Analytical Validity

"Nestin positivity or a **loss** of the expression of **inositol polyphosphate-4-phosphate (INPP4B) type 2"**: **the most strongly associated** IHC markers with **basal like** subtype

- Sensitivity (83%) and specificity (96%)

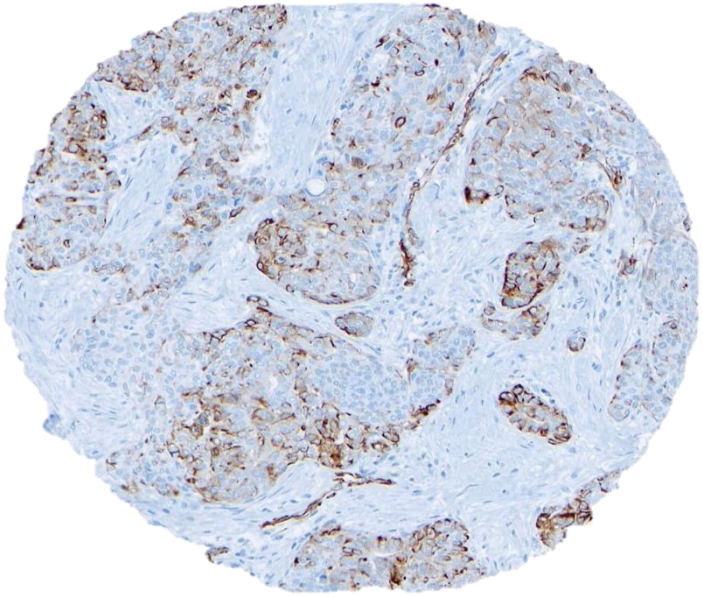
Won et al. *Mod Pathol.* 2013

Scoring of basal markers

Basal-like = Nestin+ **OR** INPP4B-

Non Basal-like = Nestin- **AND** INPP4B+

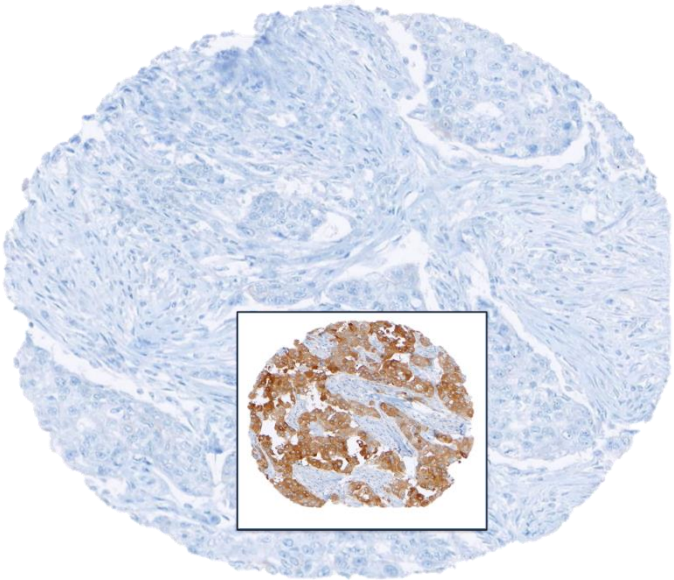
≥1%



Nestin

Parry et al. *J Clin Pathol* 2008

<5%



INPP4B

Fedele et al. *PNAS* 2010

DOI: 10.1093/jnci/djr393
Advance Access publication on September 29, 2011.

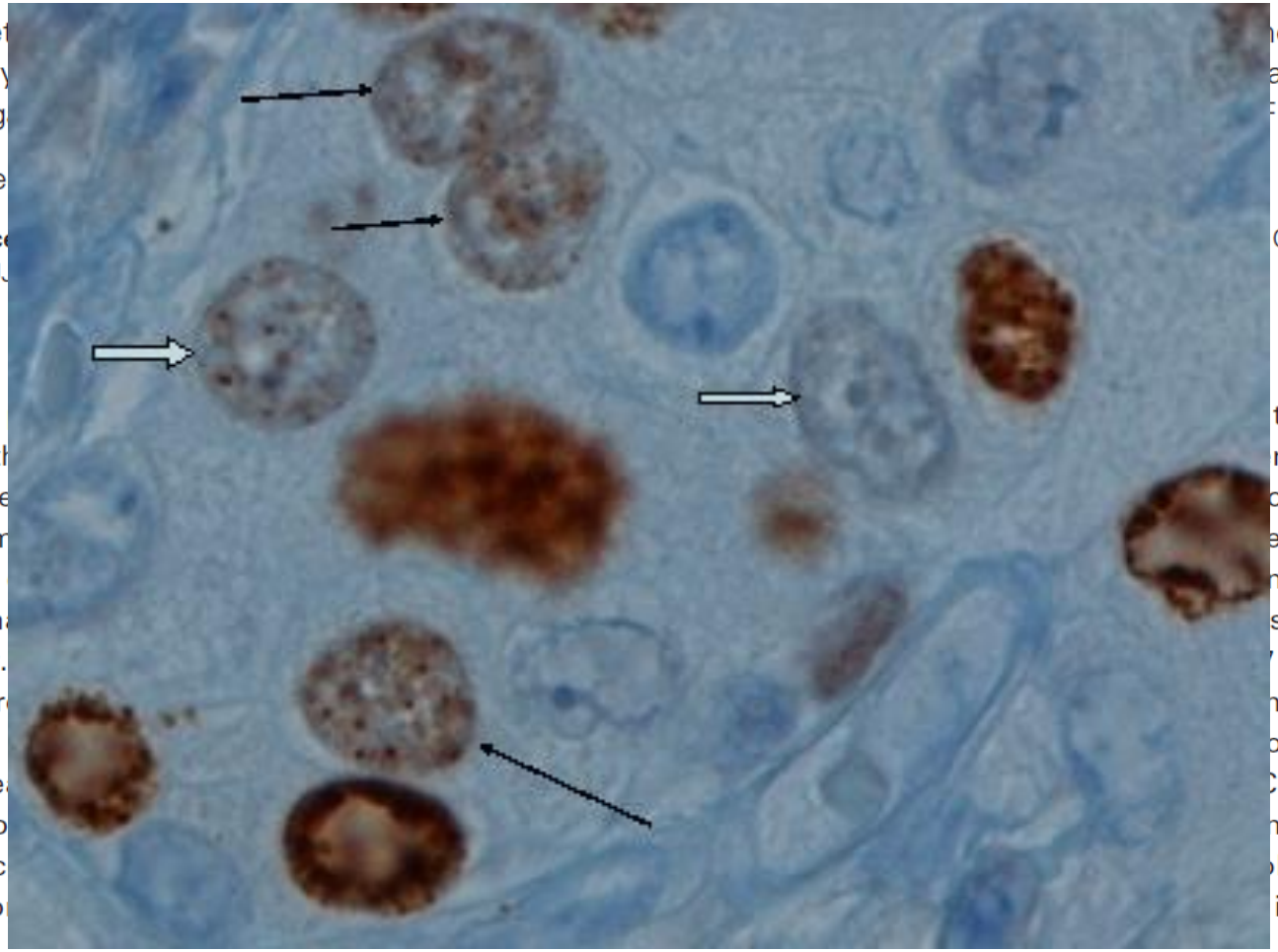
COMMENTARY

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

Mitch Dowset ew Ellis,
N. Lynn Henry a Prudkin,
Meredith Reg F. Hayes

Manuscript re

Correspondenc Cancer Centre, Fulham Rd,
London SW3 6JJ



Uncontrolled the proportion of cells staining for t ration between tumor samples. Pote otherapy or endocrine therapy, estim ent efficacy in samples taken before, ngly, Ki67 is measured in these scen sometimes for clinical management. in each of these contexts. On Mar nent of Ki67 and in the development onal Group and North American Bre Comprehensive recommendations o mulated based on current evidenc logy, create greater between-labo in clinical practice.

J Natl Cancer Inst 2011;103:1656–1664

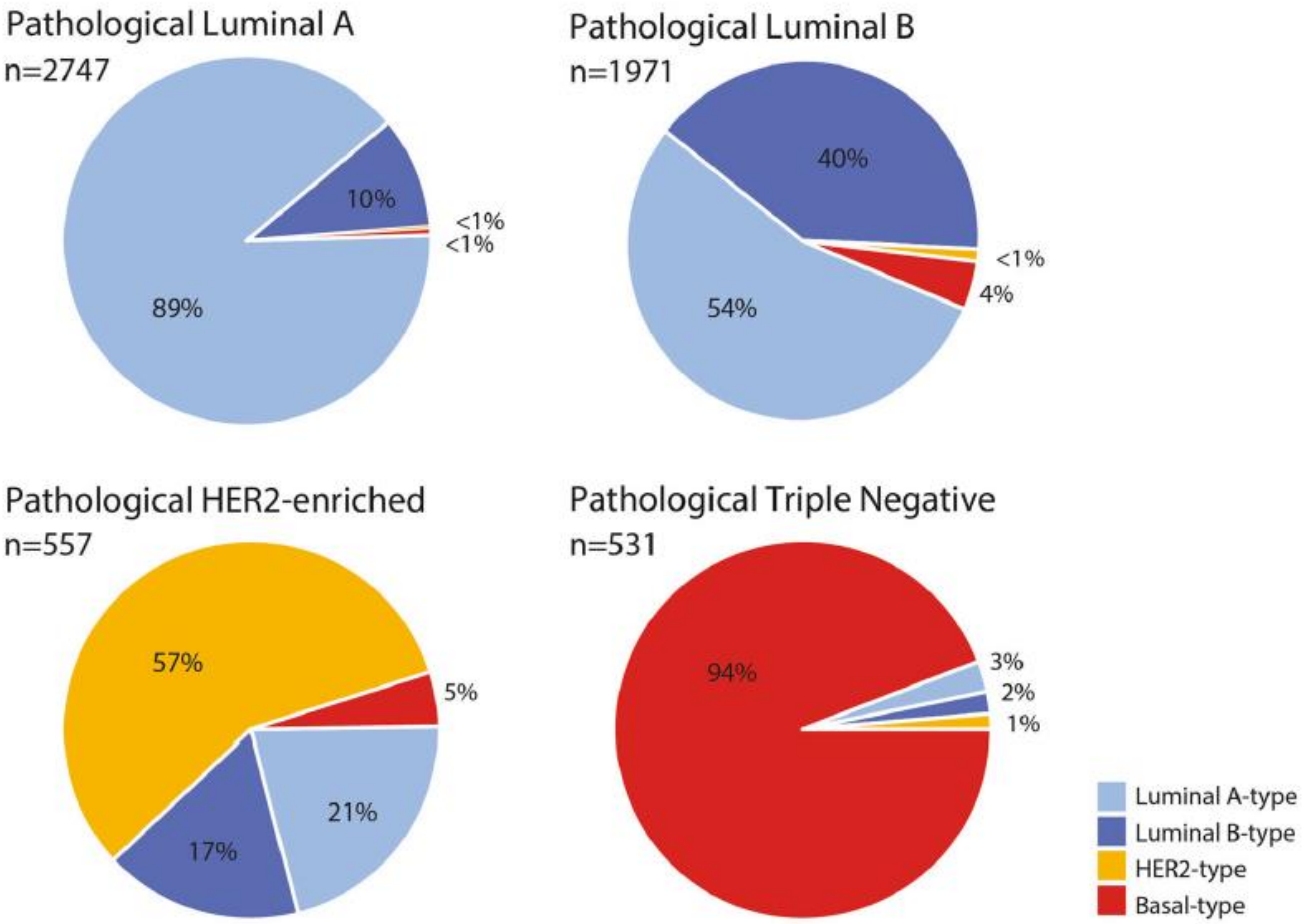
St Gallen international breast cancer conference on primary therapy of early breast cancer – the road of Ki67

Use of pathology to define intrinsic molecular breast cancer subtypes by application of IHC surrogate markers?

2009	Thresholds for therapies. Ki67: 3 categories low <15%, intermediate 16–30% and high >30%
2011	Strategies for breast cancer molecular subtypes genetic testing and attempt for approximation by surrogate IHC markers (ER, PR, HER2 and Ki67) with Ki67 cut off: 14%
2013	Personalizing the treatment of women with early breast cancer. Classification of subtypes with Luminal A: ER+, PR ≥20% and Ki67 <20%, HER2-. Luminal B: ER+ and PR<20% and/or Ki67≥20%, HER2-
2015	Tailoring therapies-improving the management of early breast cancer: Threshold value of Ki-67 within the range of 20%–29% to distinguish 'luminal B-like` subtype
2017	News since St. Gallen 2015: De-escalating and escalating treatment according to stage and breast cancer subtype: "low" ki67 versus "high" ki67

Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature.2000;406:747–752
Wirapati P et al. Meta-analysis in gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast cancr Res 2008; 10: R65
Cheang MCU, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101:736–750.
Dowsett M et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst. 2011 Nov 16;103(22)

Lack of correlation: molecular subtypes and surrogate IHC subtype classification



Viale G et al. Breast Cancer Res Treat 2017
DOI 10.1007/s10549-017-4509-9

Lack of correlation: molecular subtypes and surrogate IHC subtype classification

Table 1
Distribution of the PAM50 intrinsic subtypes within the pathology-based groups.[a]

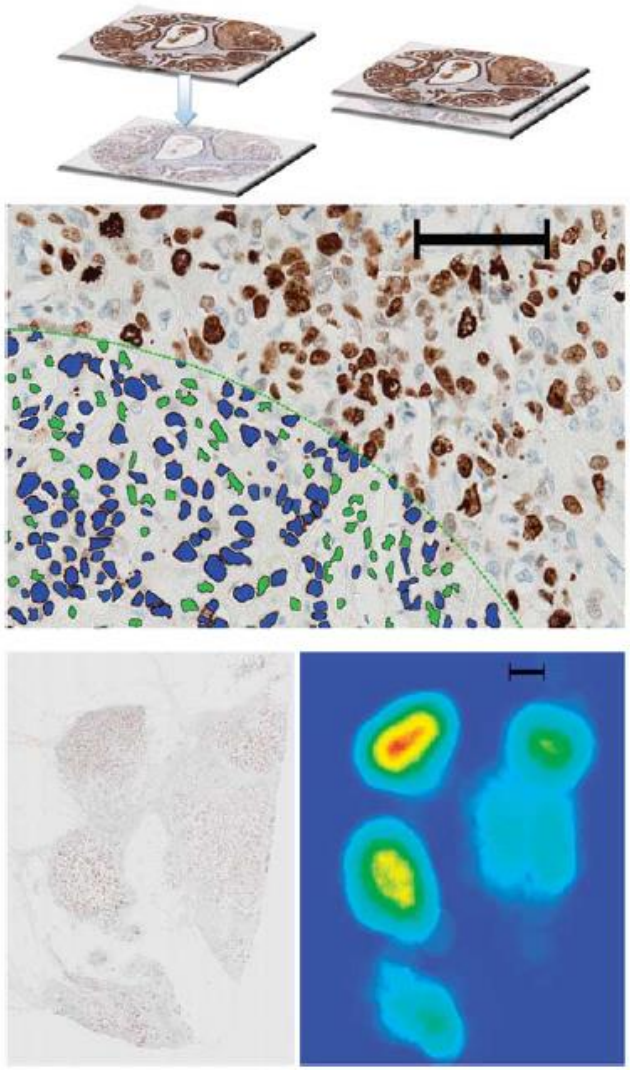
IHC-based group	References	N	PAM50 intrinsic subtype distribution			
			Luminal A	Luminal B	HER2-enriched	Basal-like
HR+/HER2−	[10,14,16–22]	4295	60.3%	31.9%	6.6%	1.2%
Luminal A	[10,14,17,21]	637	62.2%	27.0%	10.2%	0.6%
Luminal B	[10,14,17,21]	317	34.1%	51.1%	11.0%	3.8%
HER2+	[6,23–26]	831	17.6%	26.8%	44.6%	11.0%
HER2+/HR+	[25,26]	182	33.0%	46.2%	18.7%	2.2%
HER2+/HR−	[25,26]	168	19.0%	4.2%	66.1%	10.7%
TNBC	[12–15]	868	1.6%	3.2%	9.1%	86.1%

[a] The data has been obtained from the different publications. Several studies have performed a standardized version of the PAM50 assay (RT-qPCR-based or nCounter-based) from formalin-fixed paraffin-embedded tumour tissues [10,14,17,19–22], while others have performed the microarray-based version of the PAM50 assay [6,16,18,23–26].

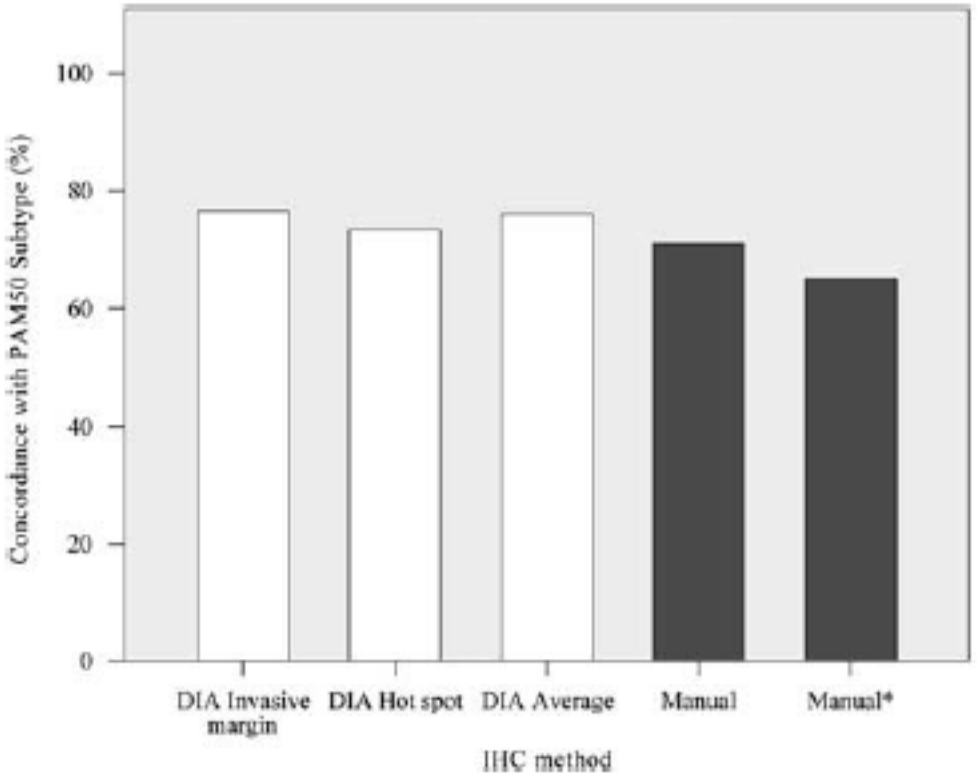
A. Prat et al. / The Breast 24 (2015) S26–S35

Digital image analysis outperforms manual biomarker assessment in breast cancer

Gustav Stålhammar[1,2], Nelson Fuentes Martinez[1,3], Michael Lippert[4], Nicholas P Tobin[5], Ida Mølholm[4,6], Lorand Kis[7], Gustaf Rosin[1], Mattias Rantalainen[8], Lars Pedersen[4], Jonas Bergh[1,5,9], Michael Grunkin[4] and Johan Hartman[1,5,7]

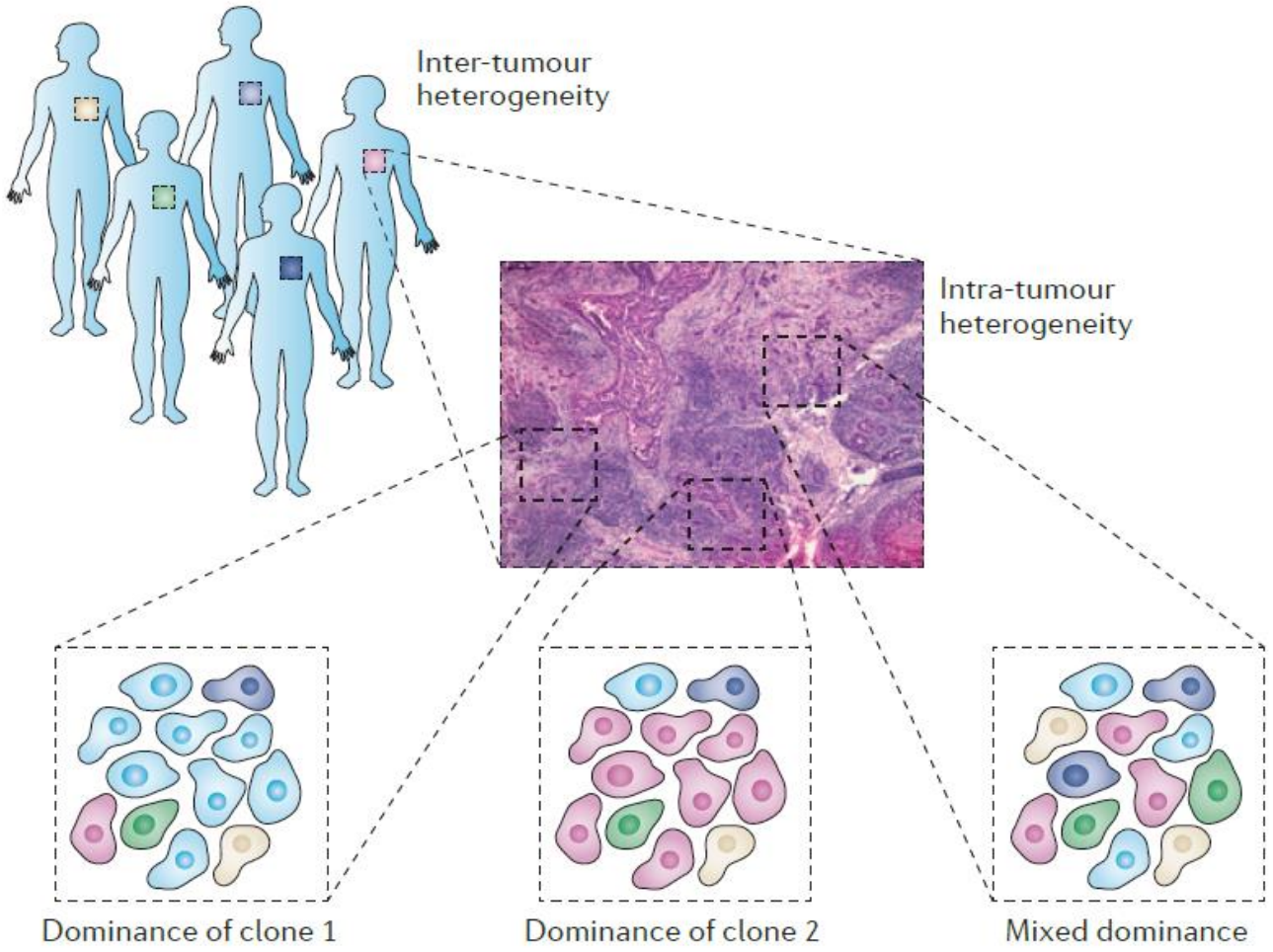


Concordance with PAM50



MODERN PATHOLOGY (2016) 29, 318–329

Tumor heterogeneity



NATURE REVIEWS
doi:10.1038/nrc3261
Published online 19 April 2012

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:
  - Treatment decision consequence in 15-20%
  -Benign disease/other malignancies in 14%

Slide courtesy of Jeanette Dupont Jensen. Department of Oncology, Odense University Hospital, Denmark

In conclusion
Immunohistochemical classification of breast tumors

- A valuable supplement for the diagnosis of ”benign versus in situ” and ”in situ versus invasive”
- Histopathological classification of malignant breast tumors
  - Treatment allocation (i.e. lobular vs non lobular)
- Prognostic and predictive factors
  - Selection of treatment and treatment duration
- Intrinsic molecular subtype / gene expression profile
  - Identification of patients who can be spared chemotherapy
- Tumor heterogeneity
  - Repeat analysis
    - multifocal tumors
    - pre/post neo-adjuvant treatment
    - primary tumour/metastasis


Thank you!