Breast cancer: IHC for diagnostic use

NordiQC Workshop in Diagnostic Immunohistochemistry 2022
Aalborg University Hospital
October 5th – 7th 2022

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Zealand University Hospital
Roskilde
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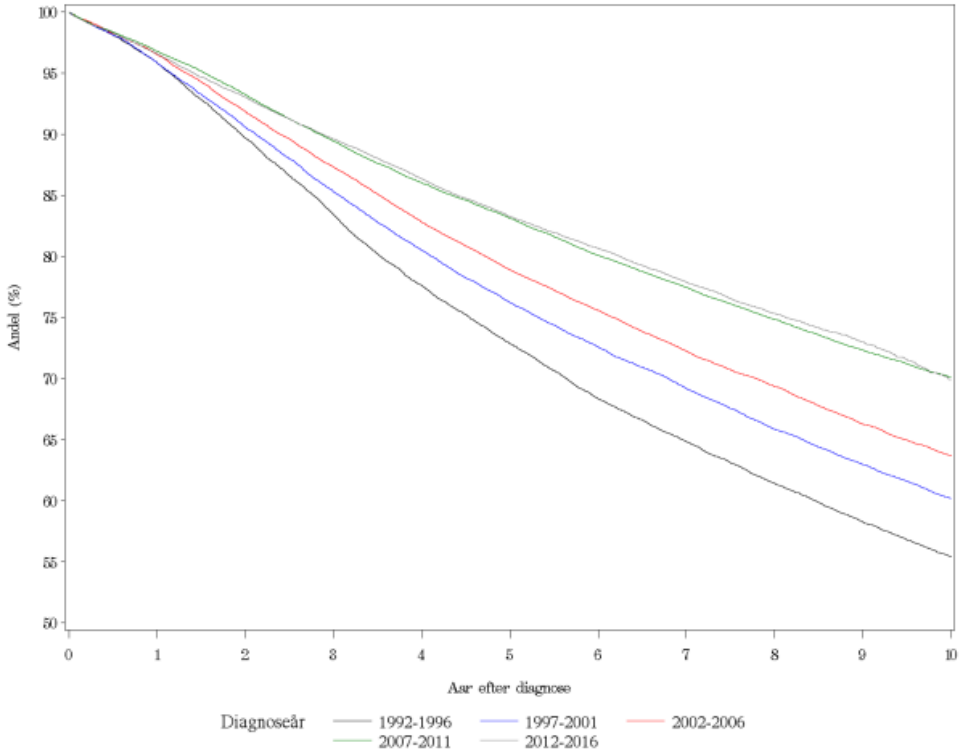
Breast cancer: 10 – year survival Denmark



Annually app 4700-5000 new cases

Overlevelse frem til 5 og 10 år efter diagnose, andel i live (95% CI)

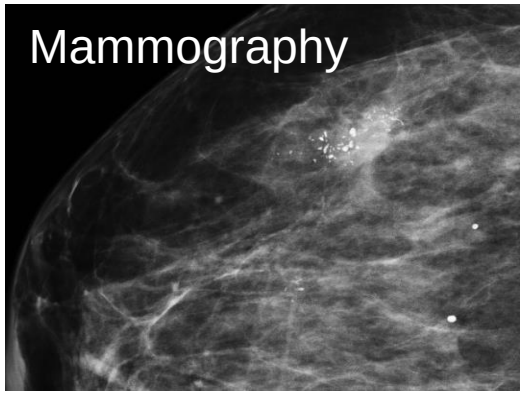
Diagnoseår	*Antal personer*	*År 5*	*År 10*
1992-1996	14279	**72,8 (72,1-73,6)**	**55,4 (54,6-56,2)**
1997-2001	16336	**76,2 (75,6-76,9)**	**60,2 (59,4-60,9)**
2002-2006	18149	**78,9 (78,3-79,5)**	**63,7 (62,9-64,4)**
2007-2011	22992	**83,1 (82,6-83,6)**	**70,1 (69,5-70,7)**
2012-2016	22785	**83,3 (82,8-83,7)**	**70,0 (69,0-70,8)**

DBCG Kvalitetsdatabase for Brystkræft · National årsrapport 2021
RKKP's Videncenter · www.rkkp.dk

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 and *HER2 low status*
    - Ki67
    - PD-L1
  - Molecular subtypes

Triple Test
Diagnostic approach – Breast Tumours



Physical breast exam/

Palpation

Radiology

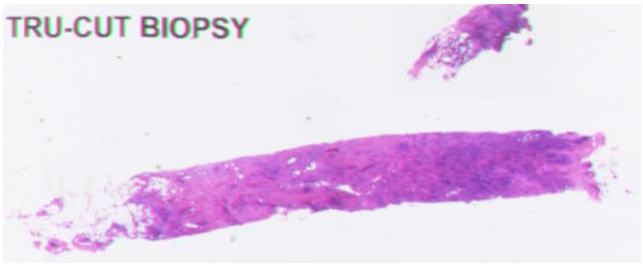
Mammography

Ultrasound

Pathology

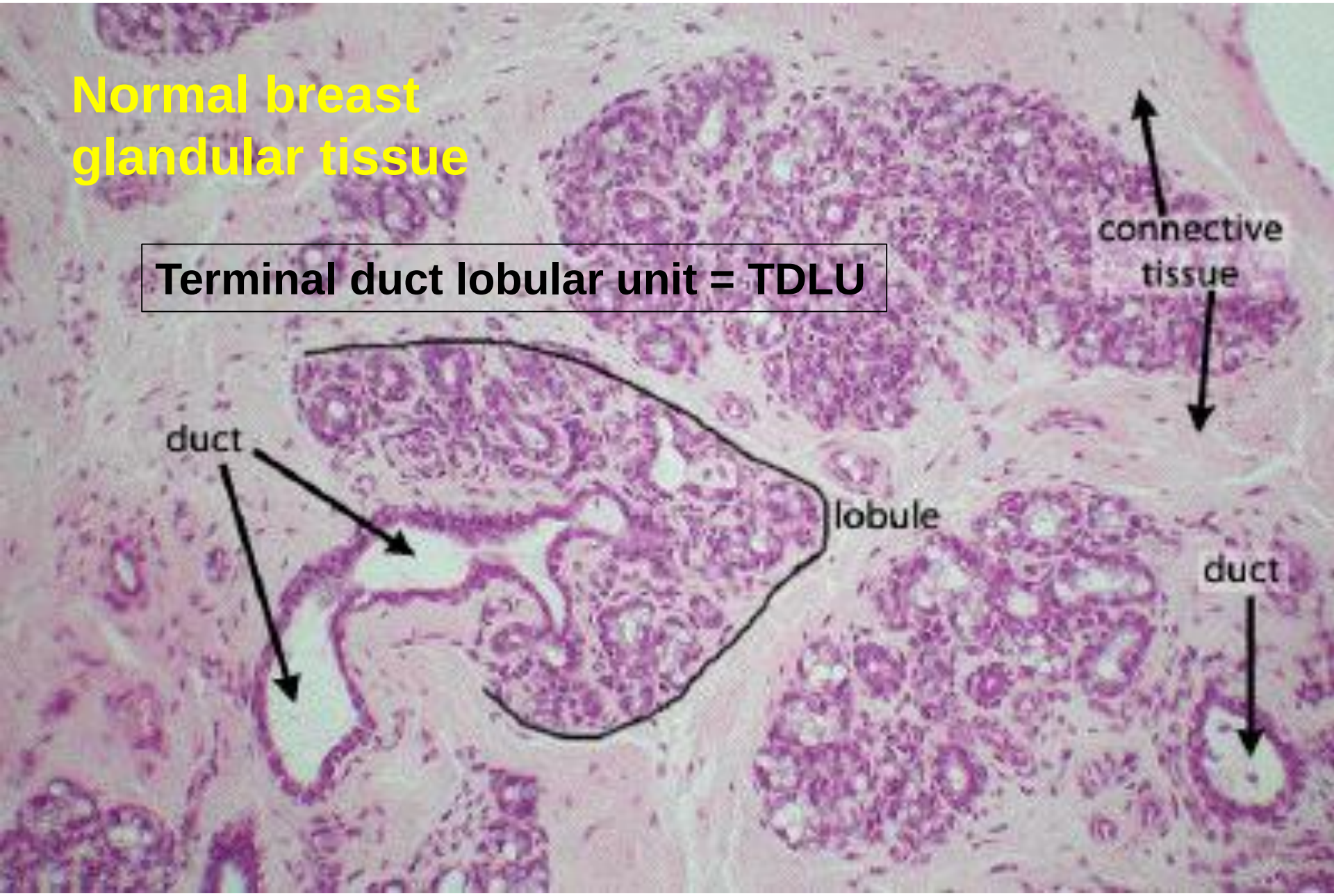
Core needle biopsy or Fine needle aspiration

Triple diagnostics



Normal breast glandular tissue

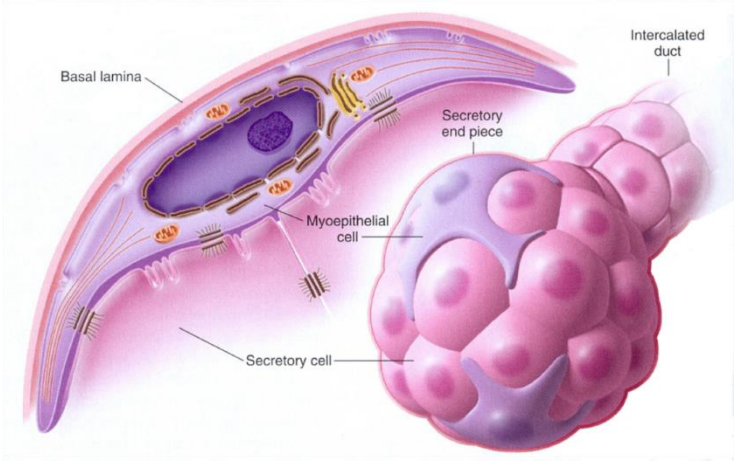
Terminal duct lobular unit = TDLU



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

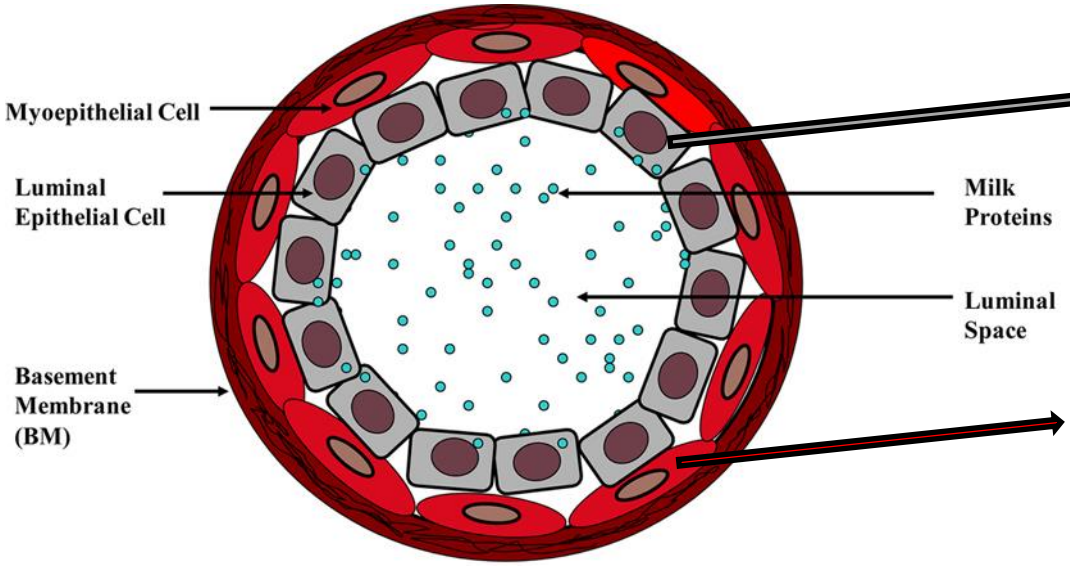
Luminal cell

Myoepithelial cell



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

Epithelial cells with specific immunohistochemical phenotype

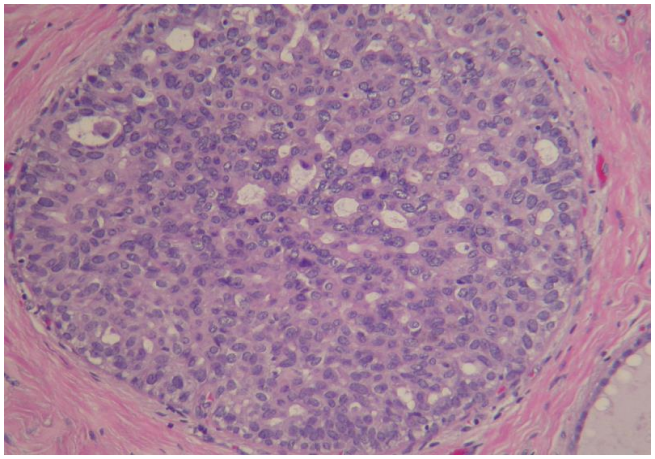


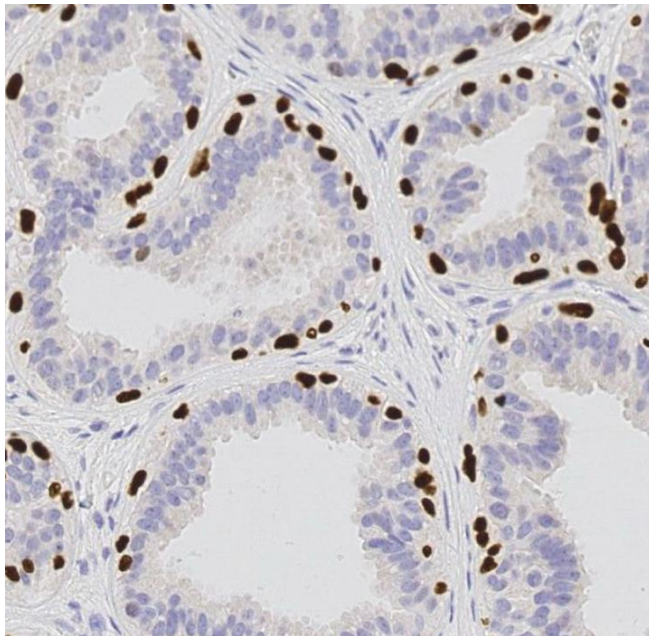
Luminal markers (LMW):
CK7, CK8, CK18, CK19

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

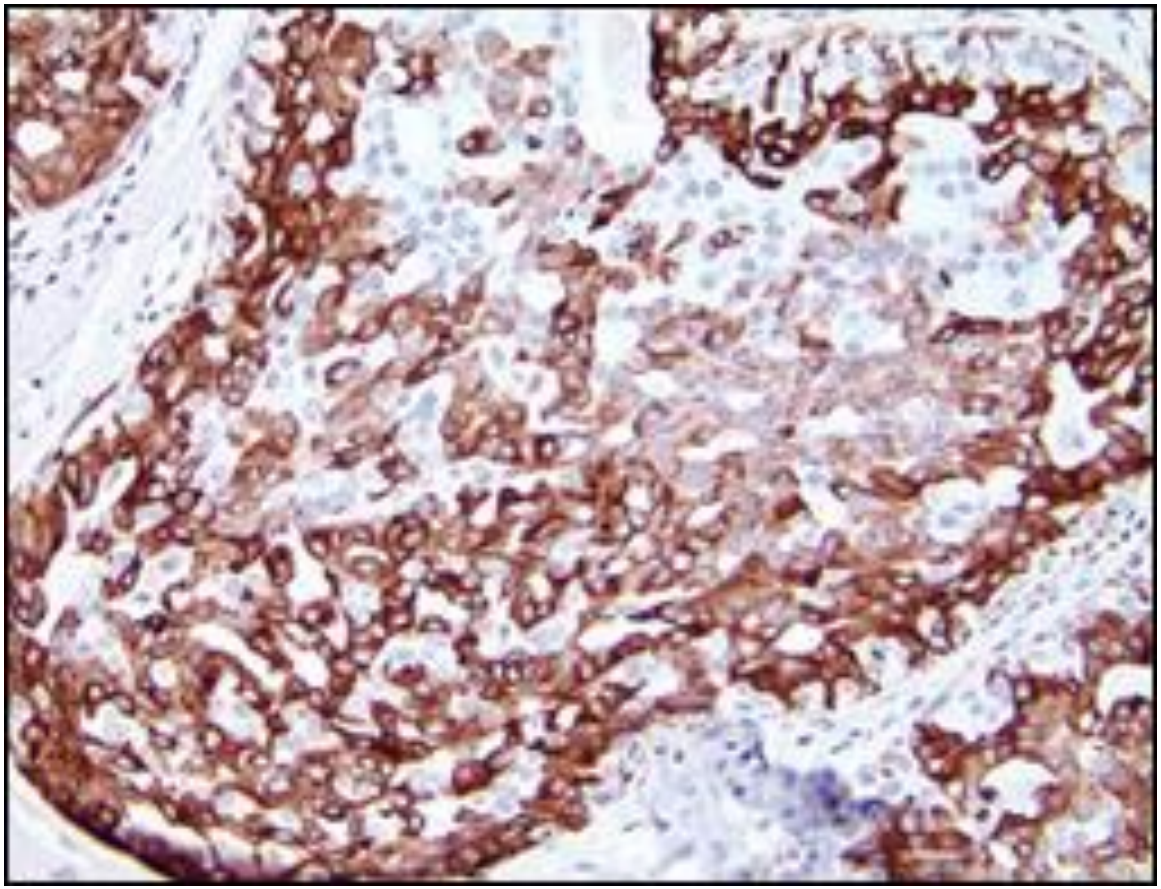
*Smooth muscle myosin heavy chain

Benign hyperplasia
Positive staining for myoepitelial cells





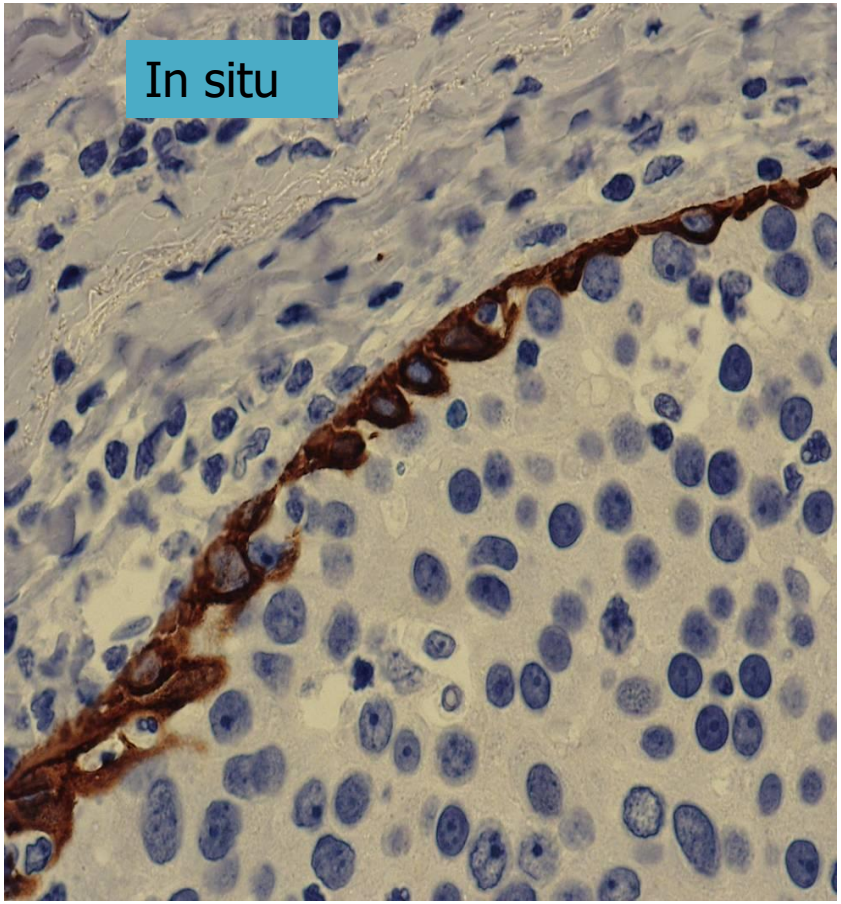
P63



CK5

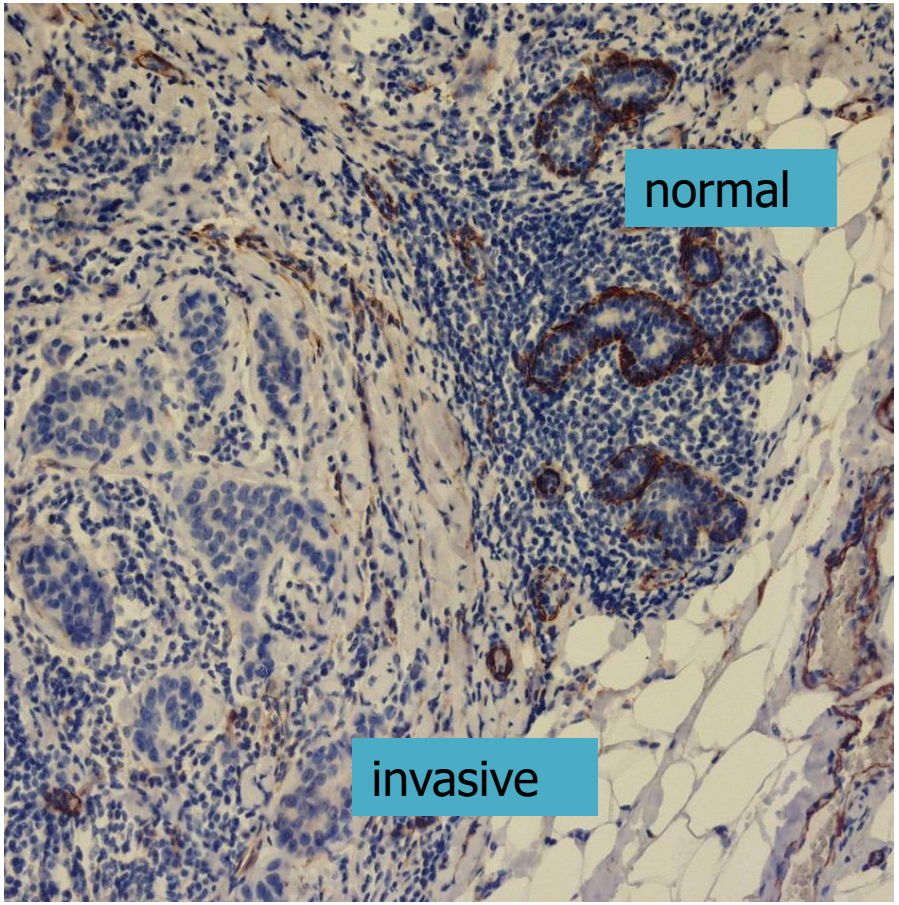
Differentiation between ductal carcinoma in situ and Invasive Carcinoma i.e. SMMHC*

present



Detecting ”presence”

Not present

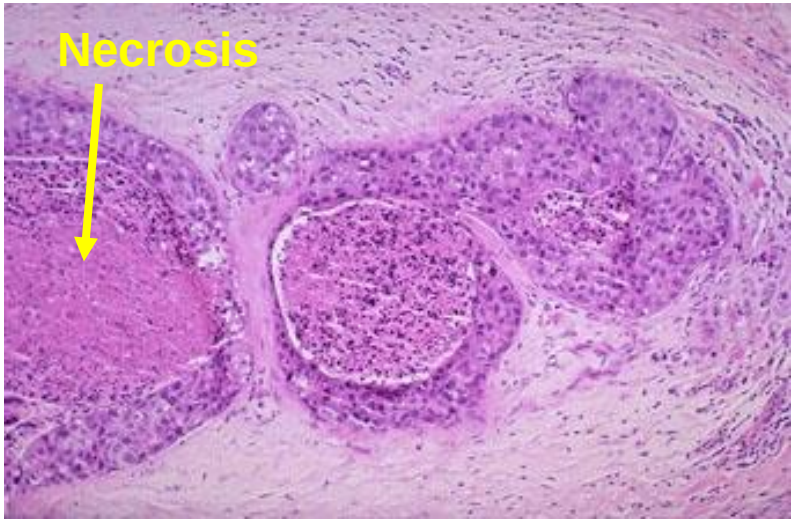


Detecting ”absence”

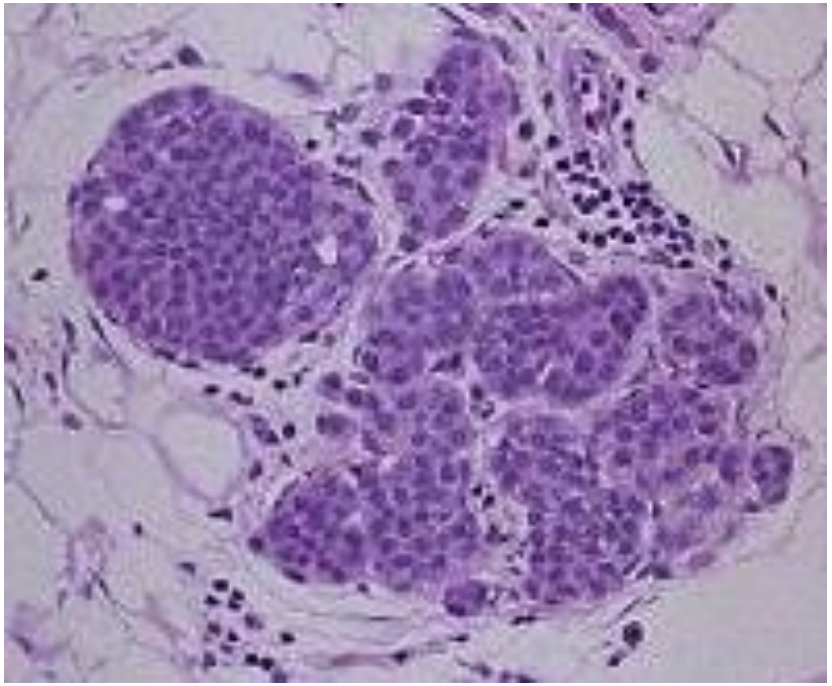
* Smooth muscle myosin heavy chain, as detected with clone SMMS-1

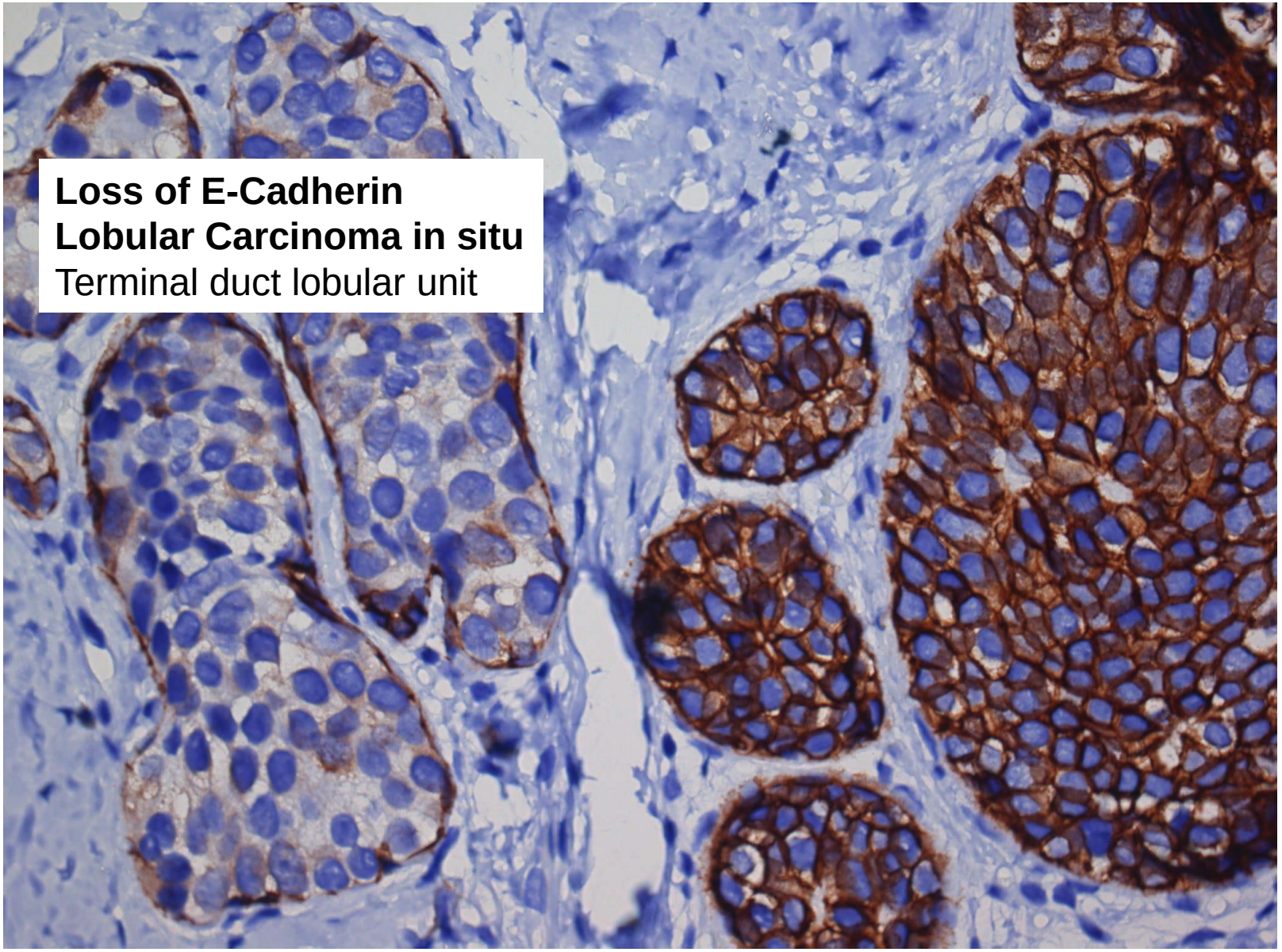
Carcinoma in situ

- 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 (2 mm)
  - Radiation therapy after breast conserving surgery



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





E-cadherin: Cell Adhesion Molecule

Classification of malignant tumors of the breast

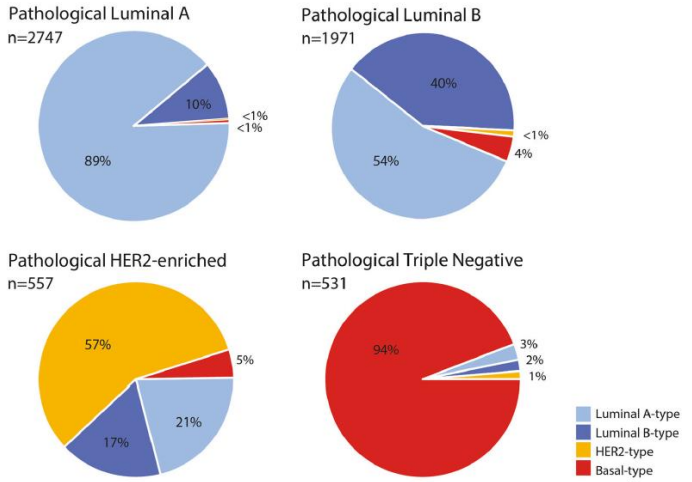
WHO blue books

Histological subtypes

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

Intrinsic molecular subtypes

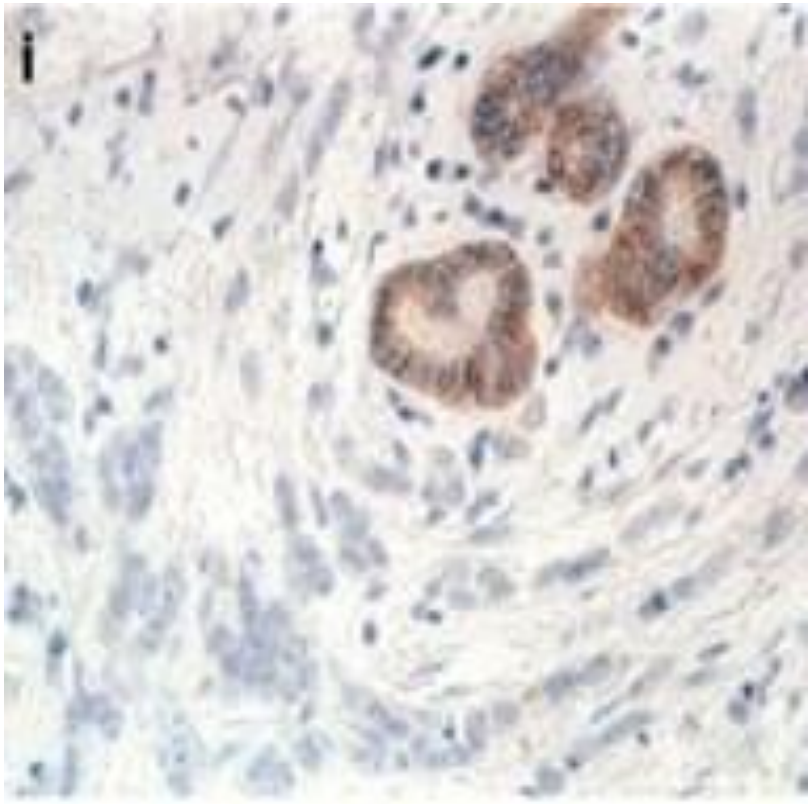
- **Luminal A**: ER+, low proliferative
- **Luminal B**: ER+, high(er) proliferative, (HER2+)
- **HER2 Enriched:** (HER2 positive)
- **Basallike:** (ER-, PR- HER2-)



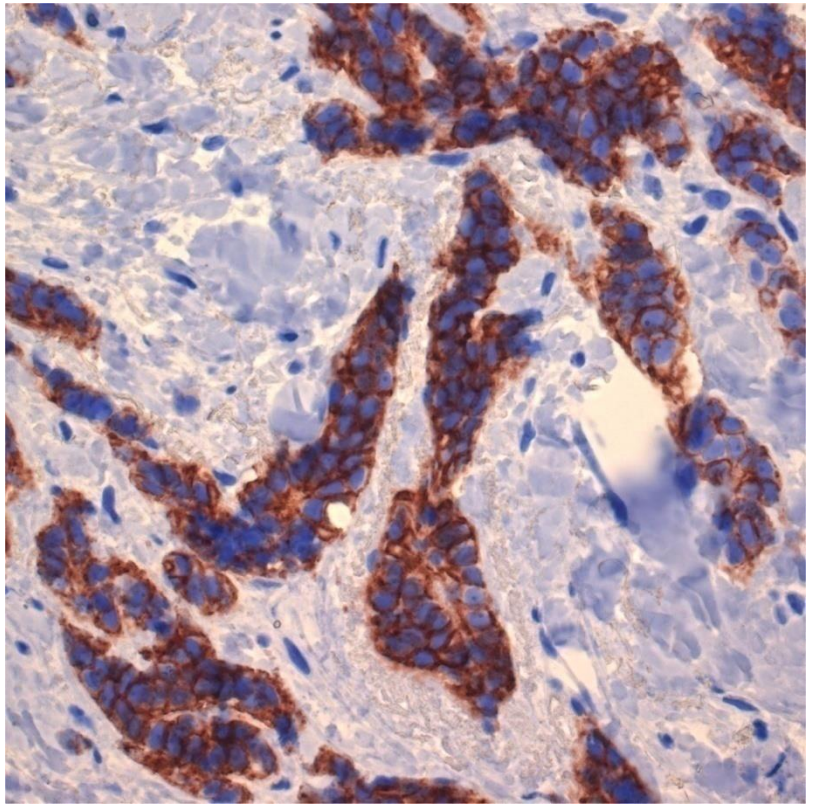
Lack of correlation between IHC subtype and molecular subtype

E-Cadherin
Cell adhesion molecule

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

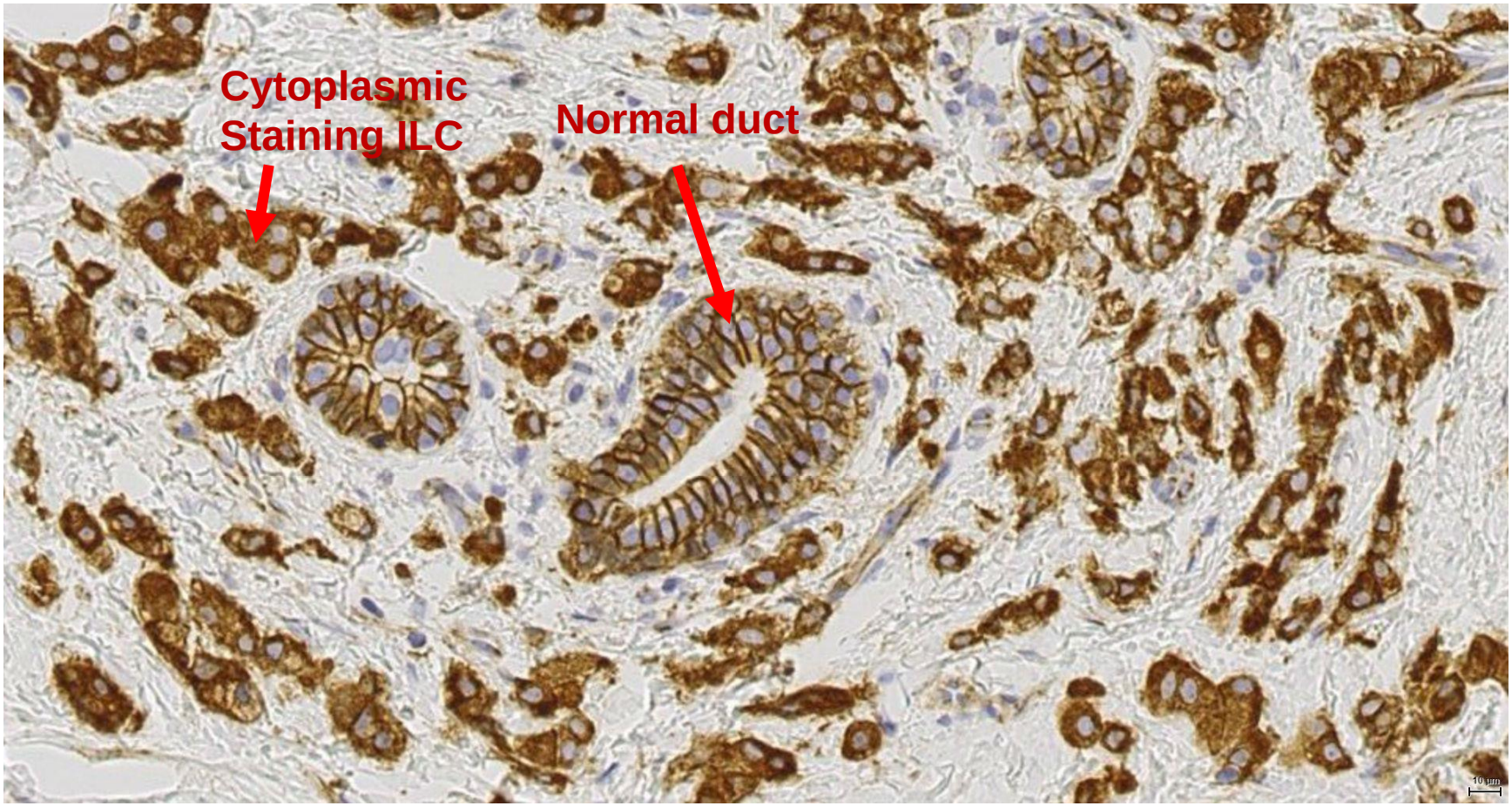


E-Cadherin positive Invasive Ductal Carcinoma



CDH1 (16q22.1) loss of function mutation or deletion resulting in loss of the adhesion molecule E-cadherin

P120 catenin dislocated to the cytoplam in lobular carcinoma (ILC)
A supplement for classification of lobular neoplasia



Lobular cancer - not candidate for neoadjuvant chemotherapy
Low proliferating tumors, often luminal A molecular subtype

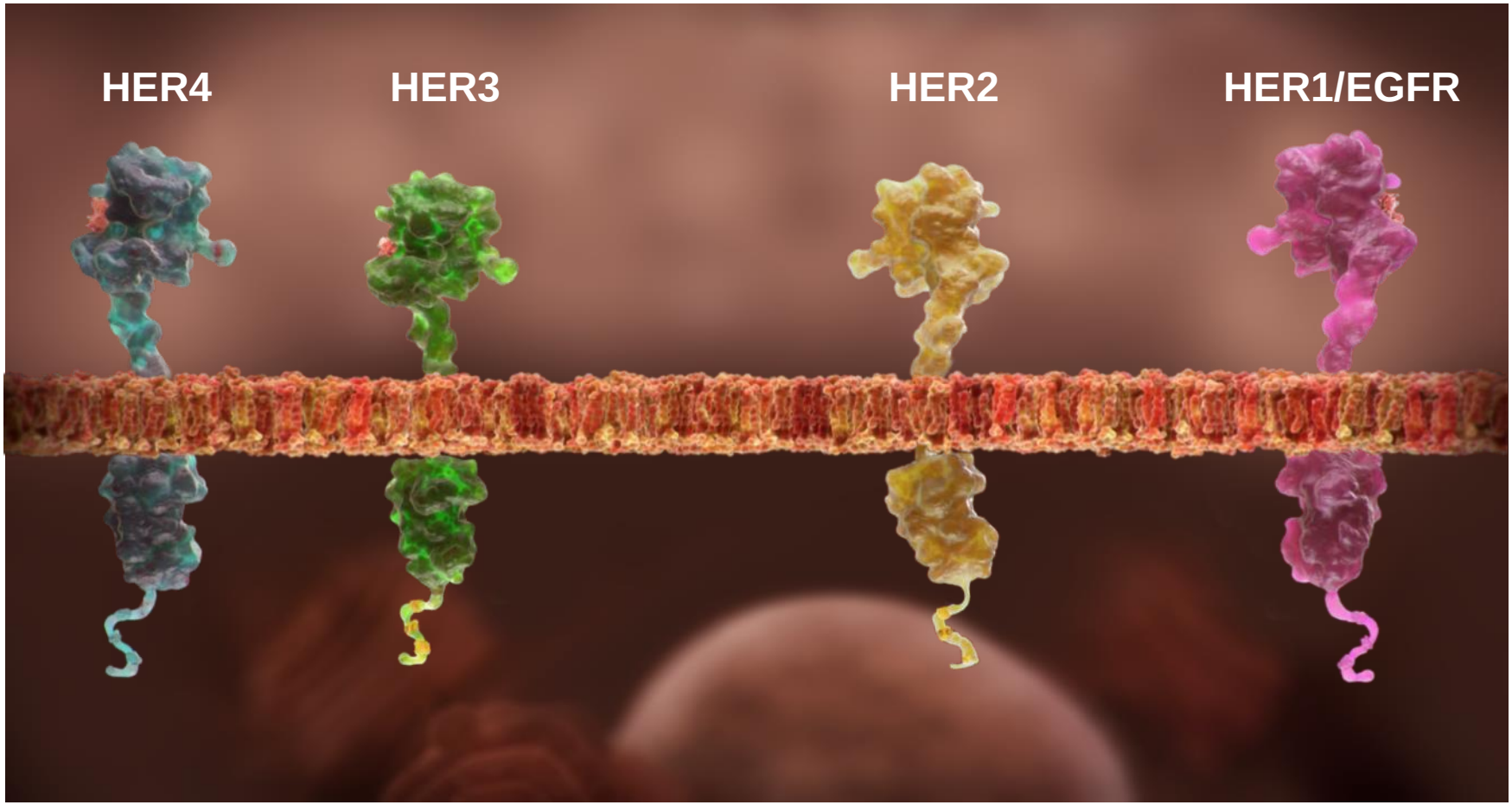
Prognostic and predictive biomarkers

HER2 positive breast cancer: 12%

Family of four receptors in the HER family

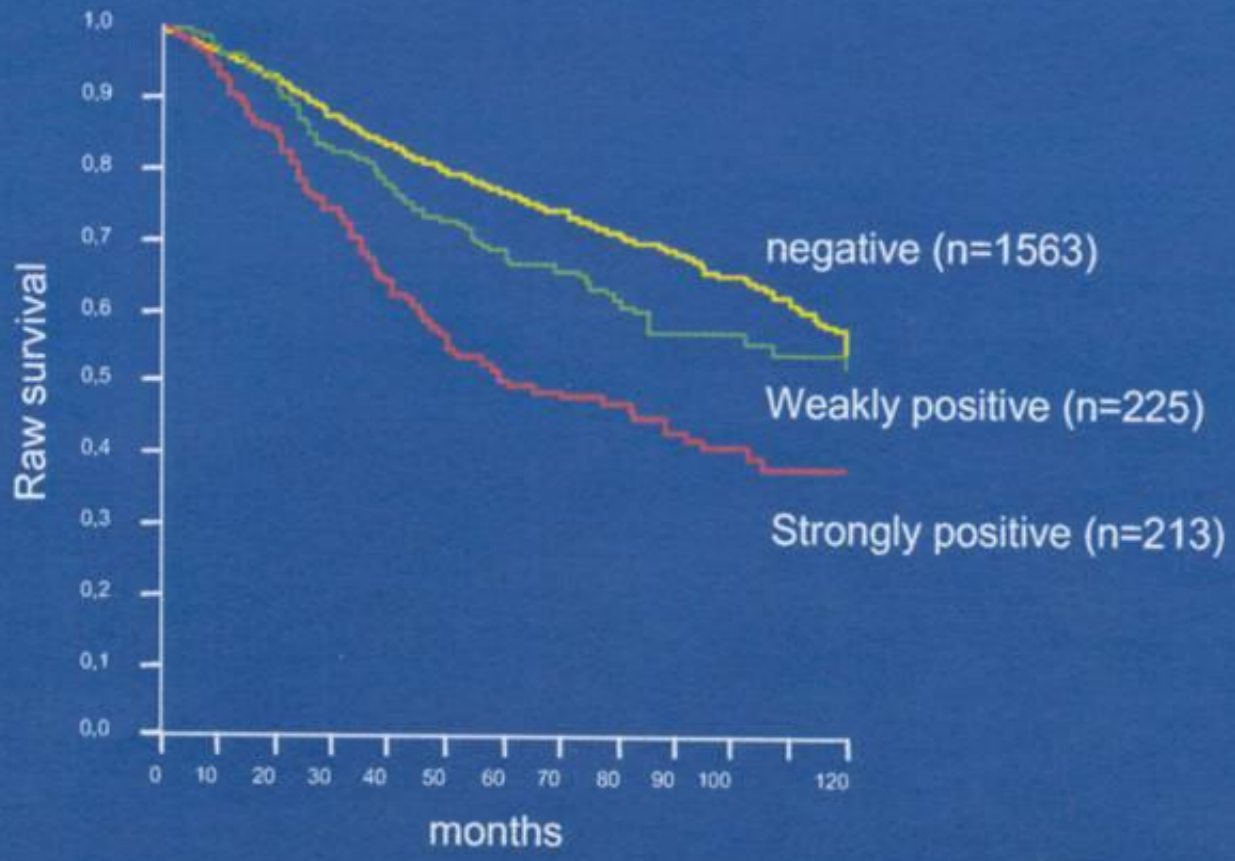
HER2: Growth factor tyrosine kinase receptor

Mediate cell growth differentiation and survival



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

HER2 and Breast Cancer Progression



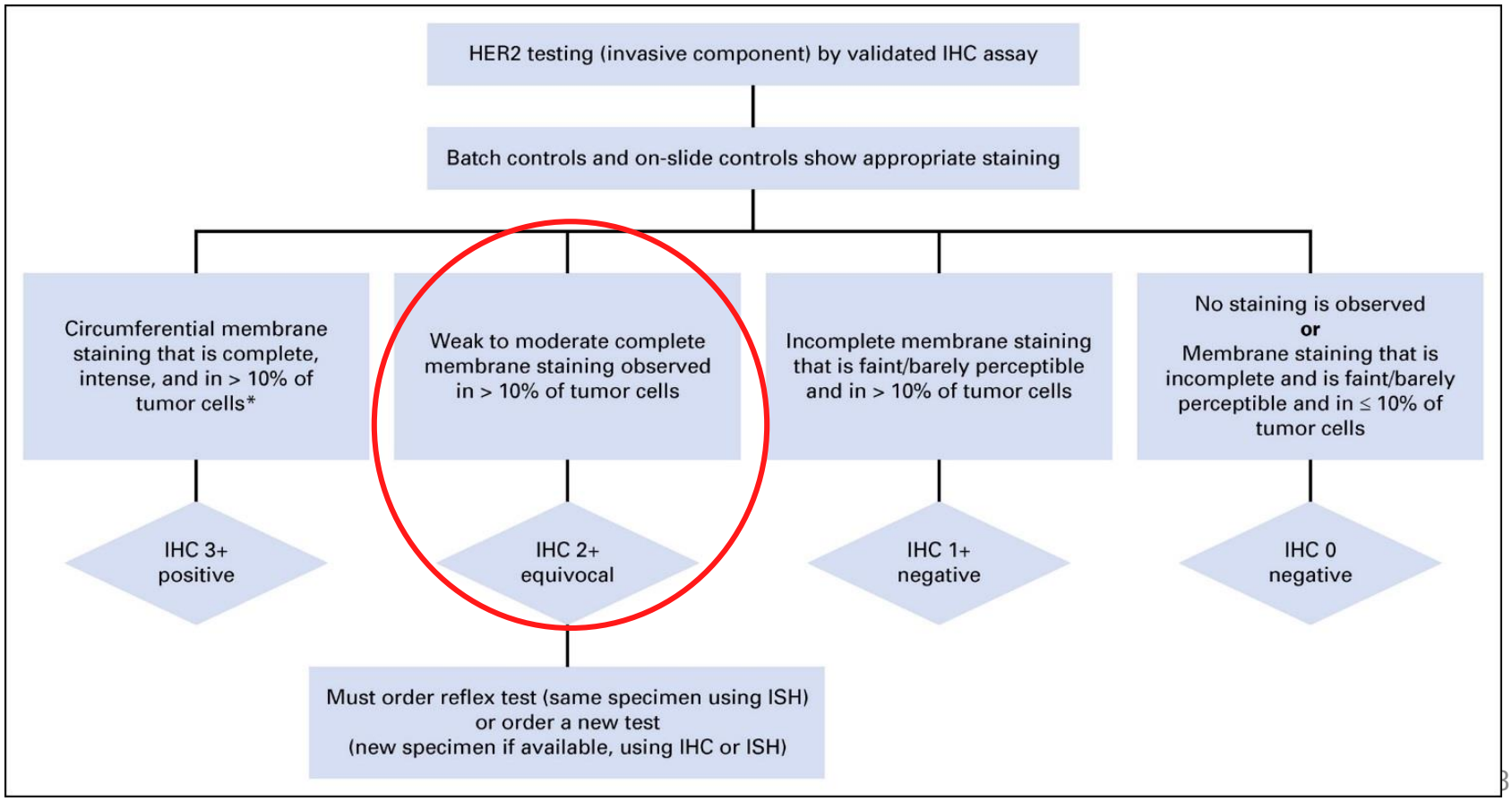
Science, Vol 235, 1987

VOLUME 36 · NUMBER 20 · JULY 10, 2018

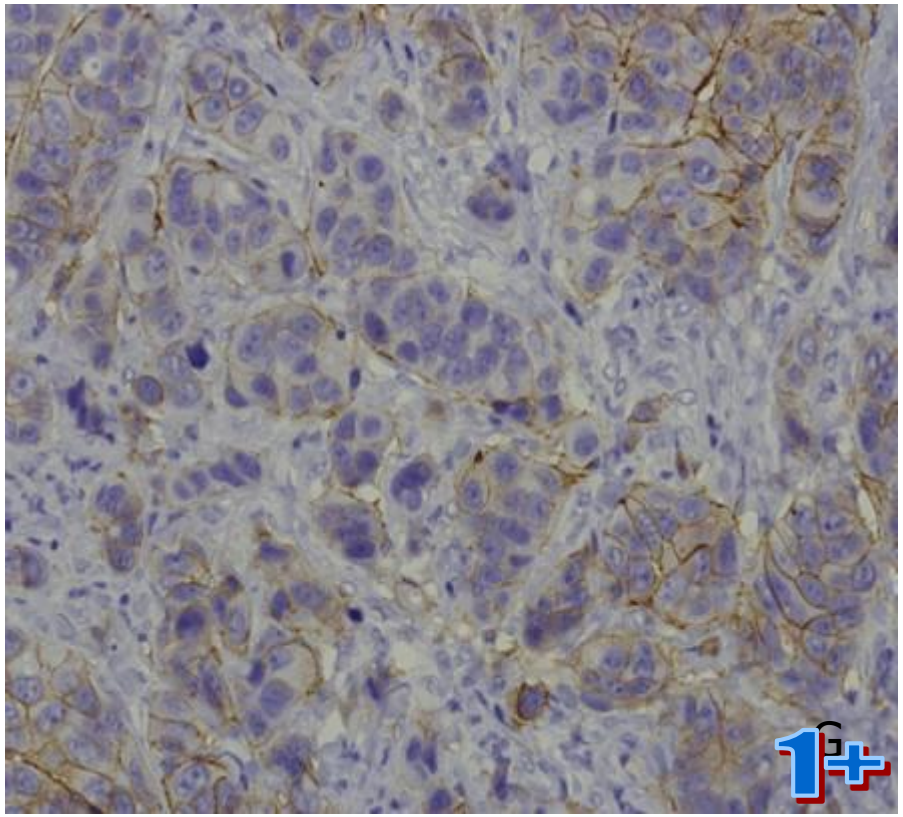
JOURNAL OF CLINICAL ONCOLOGY

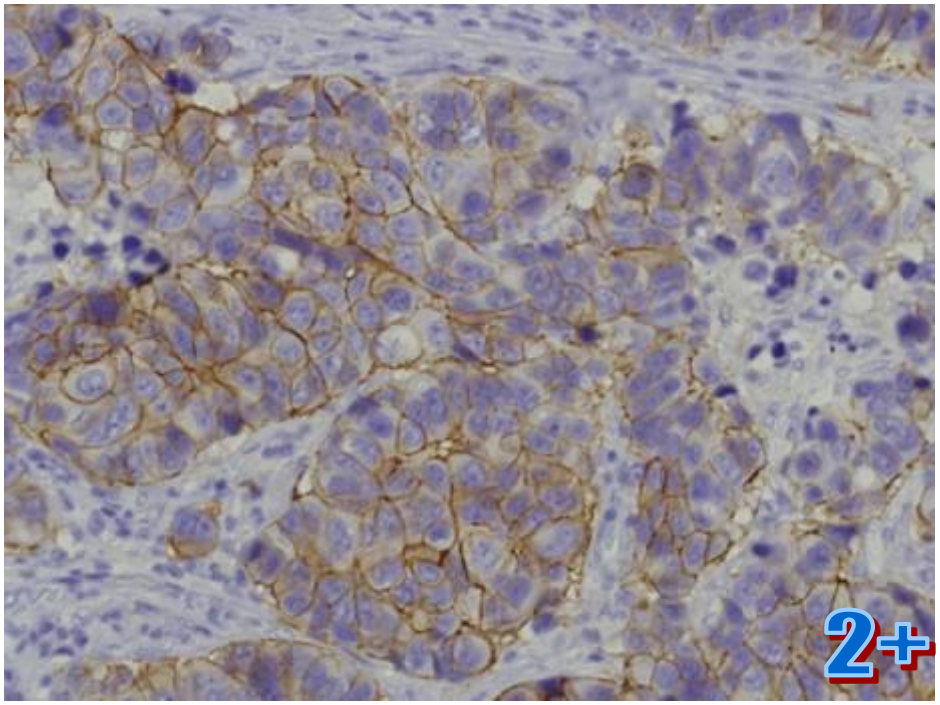
ASCO SPECIAL ARTICLE

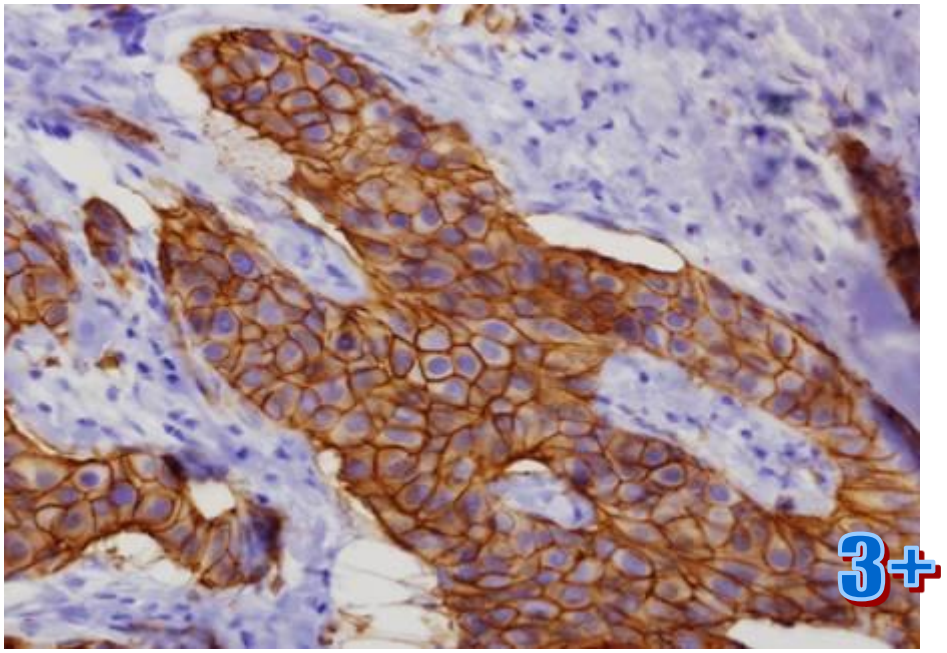
Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update



HER2 IHC



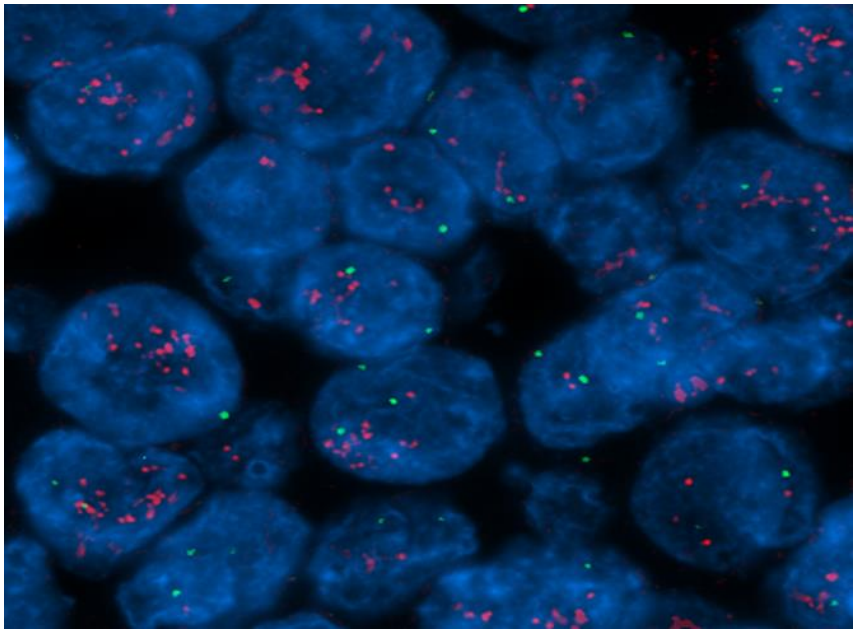




HER2 3+ and ISH + : 12 % (DK)

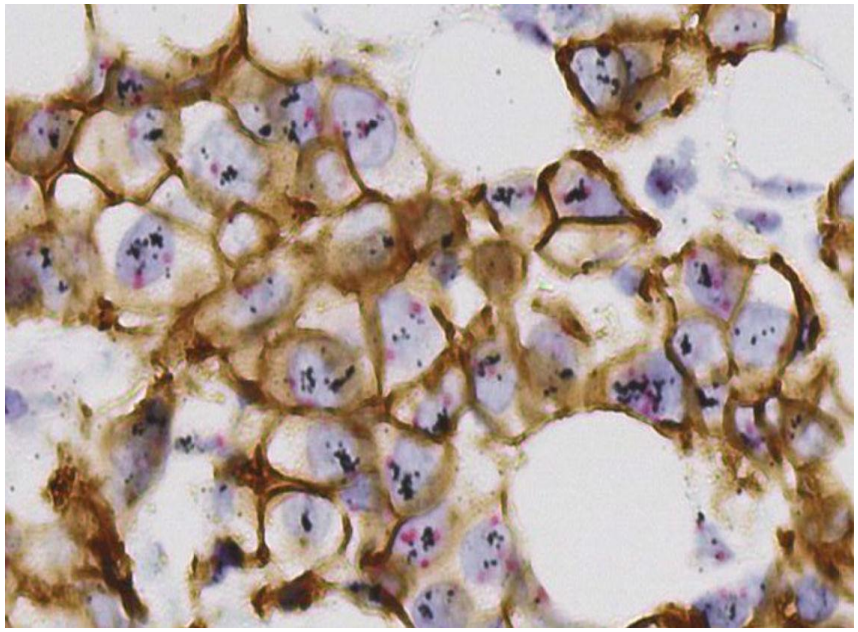
HER2 dual probe (F)ISH assay

FISH



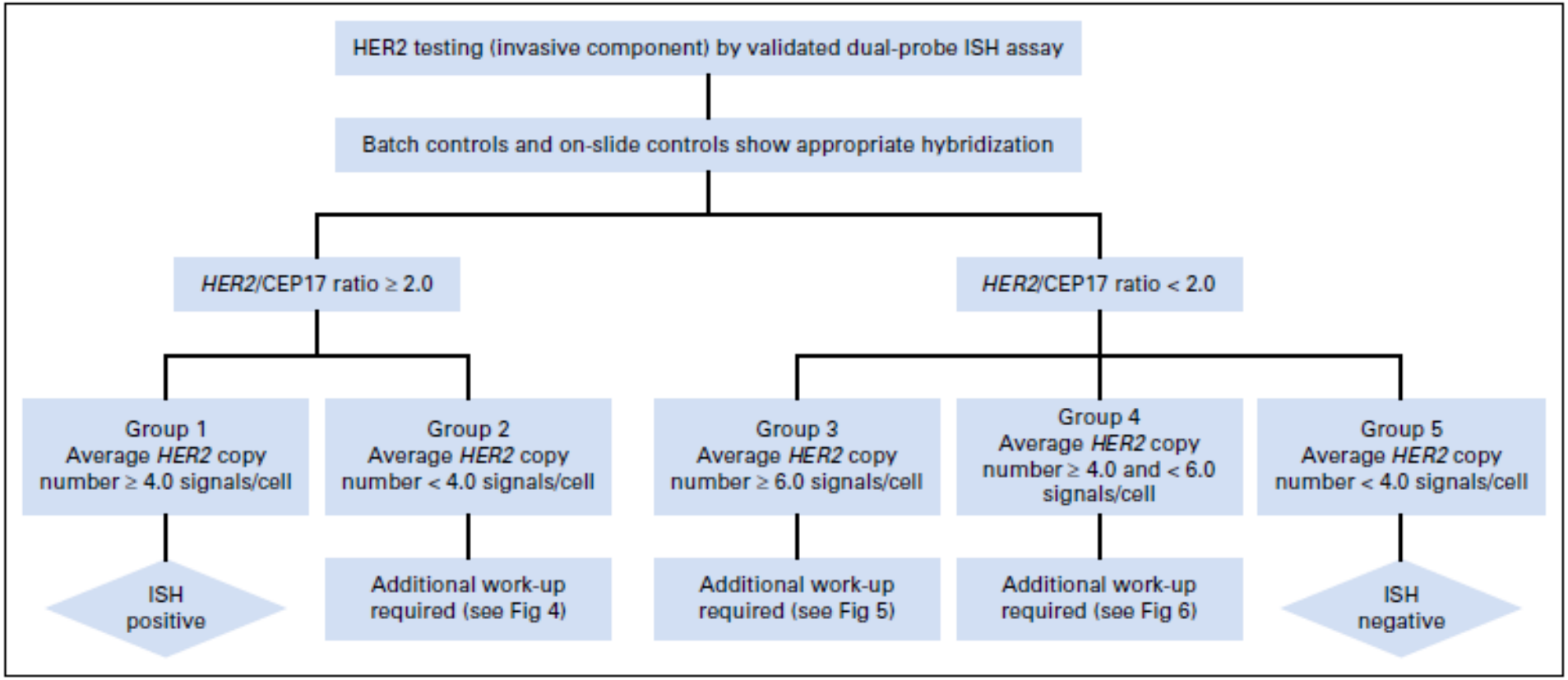
Red: HER2 gene
Green: Centromere region/chromosome 17
HER2 amplified ratio > 2

HER2 Gene/Protein Assay

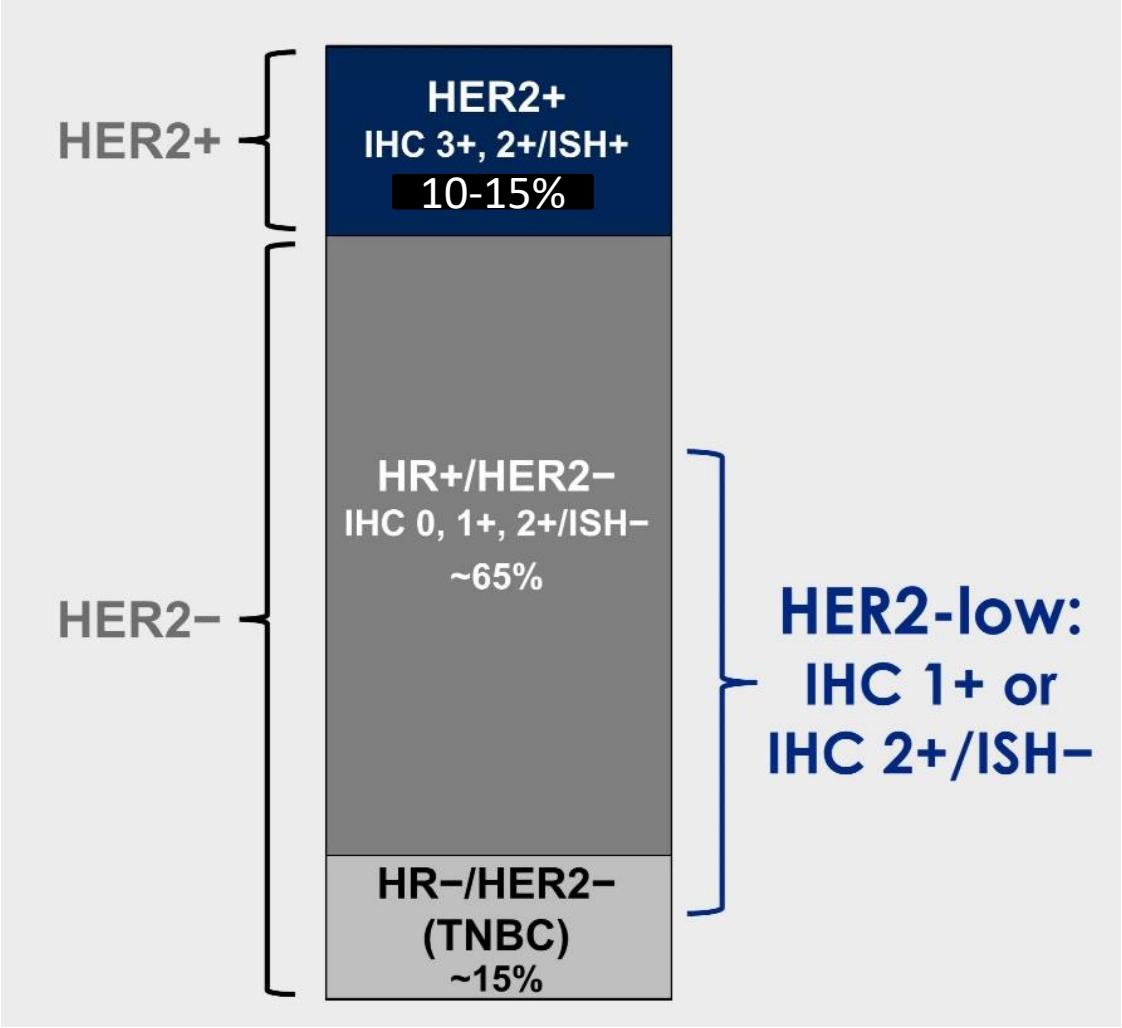


Black: HER2 gene
Red: Centromere region/chromosome 17
HER2 amplified ratio > 2 and HER2 IHC 3+

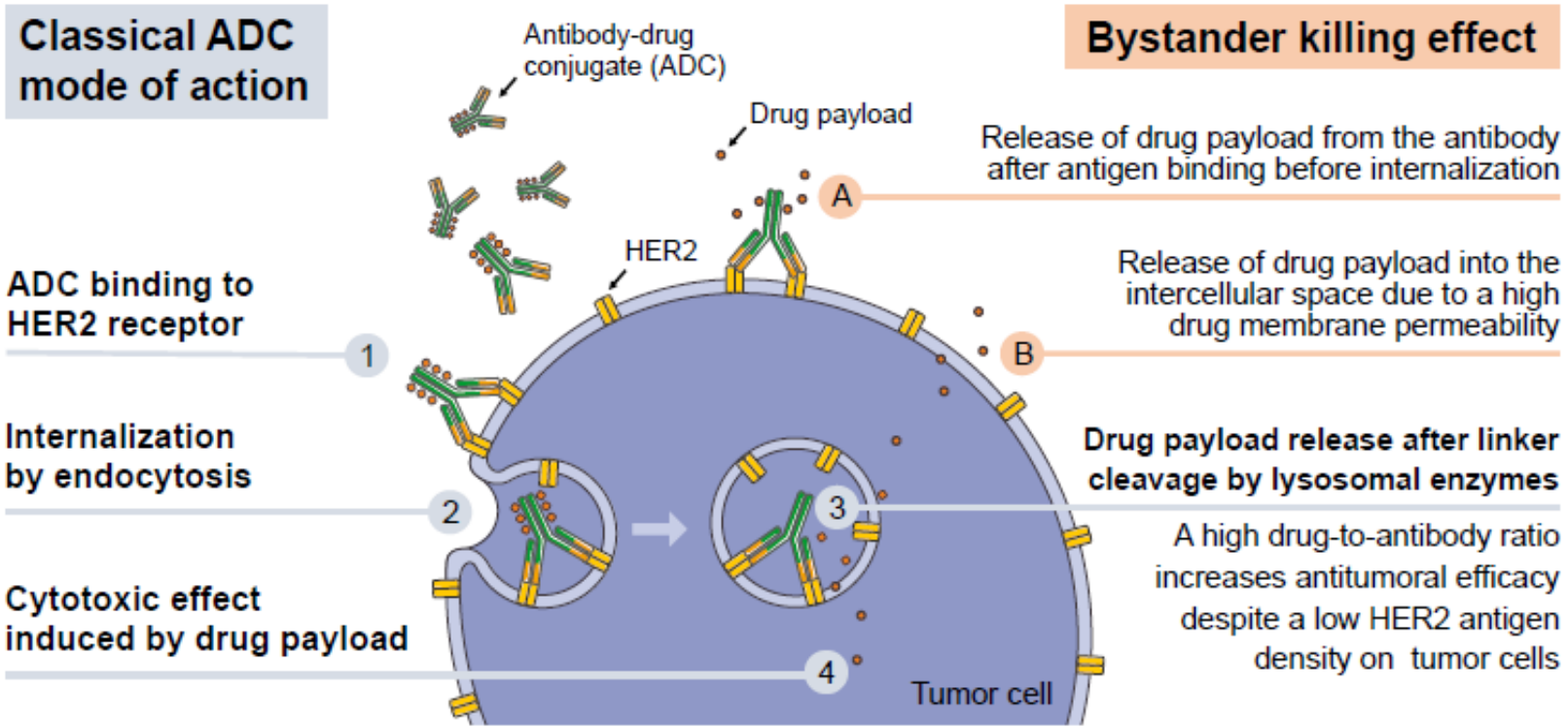
HER2 testing by validated dual-probe ISH assay



HER2 Low – a new entity for targeted treatment (metastatic disease)



HER2 Low – a new entity for targeted treatment - ADC



Int. J. Mol. Sci. **2019**, *20*, 1115

Modi et al. JCO 2020

ADC= Anti-body-drug conjungates

Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer

DESTINY-Breast04: First Randomized Phase 3 Study of T-DXd for HER2-low mBC

An open-label, multicenter study (NCT03734029)

Patients[a]
- HER2-low (IHC 1+ vs IHC 2+/ISH−), unresectable, and/or mBC treated with 1-2 prior lines of chemotherapy in the metastatic setting
- HR+ disease considered endocrine refractory

R 2:1

T-DXd
5.4 mg/kg Q3W
(n = 373)

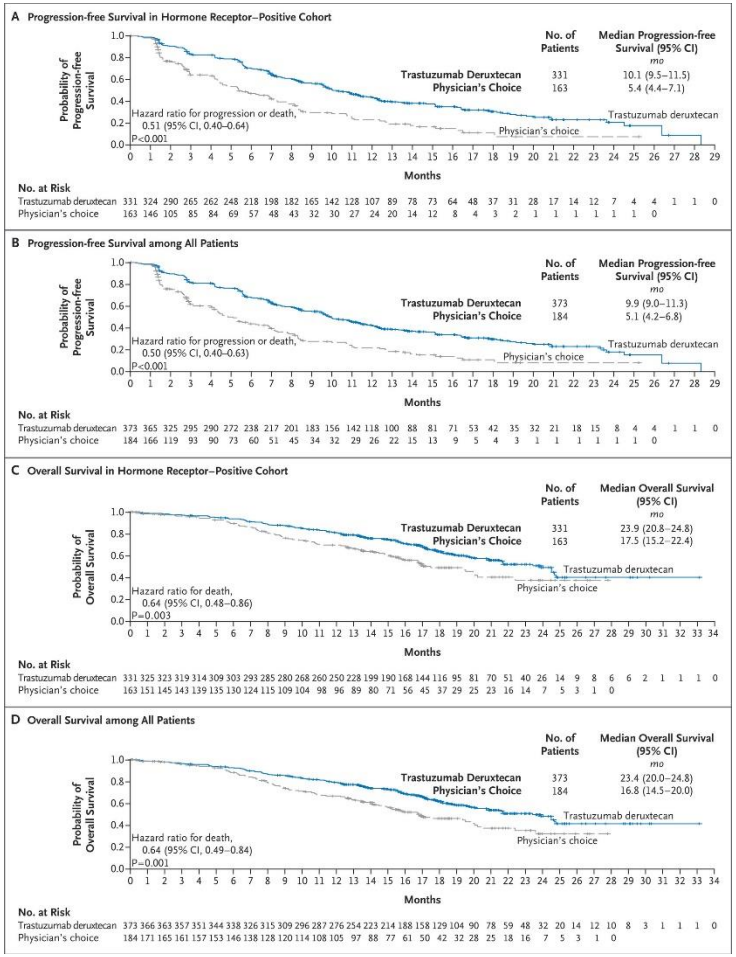
HR+ ≈ 480
HR− ≈ 60

TPC
Capecitabine, eribulin, gemcitabine, paclitaxel, nab-paclitaxel[c]
(n = 184)

Primary endpoint
- PFS by BICR (HR+)

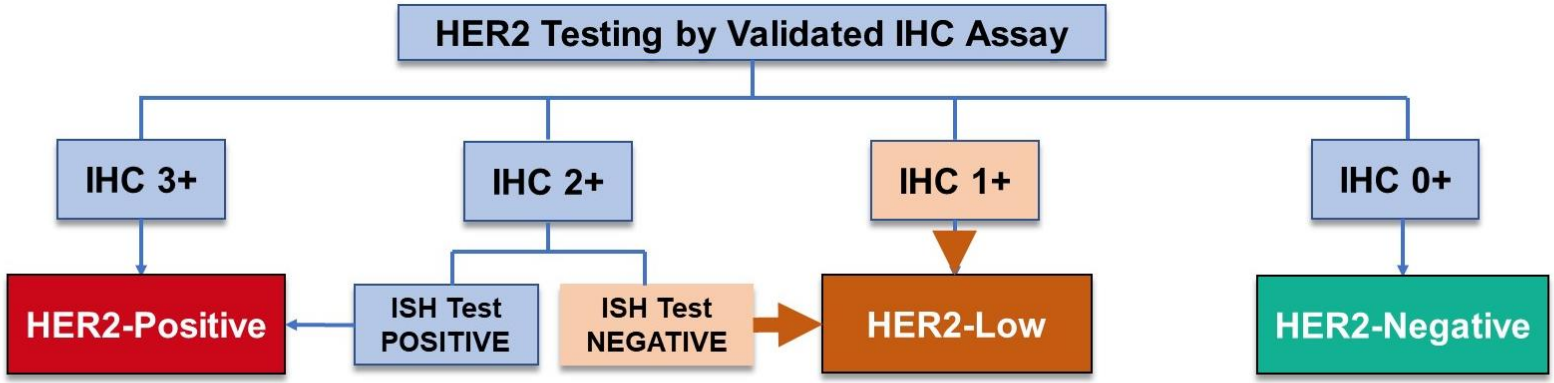
Key secondary endpoints[b]
- PFS by BICR (all patients)
- OS (HR+ and all patients)

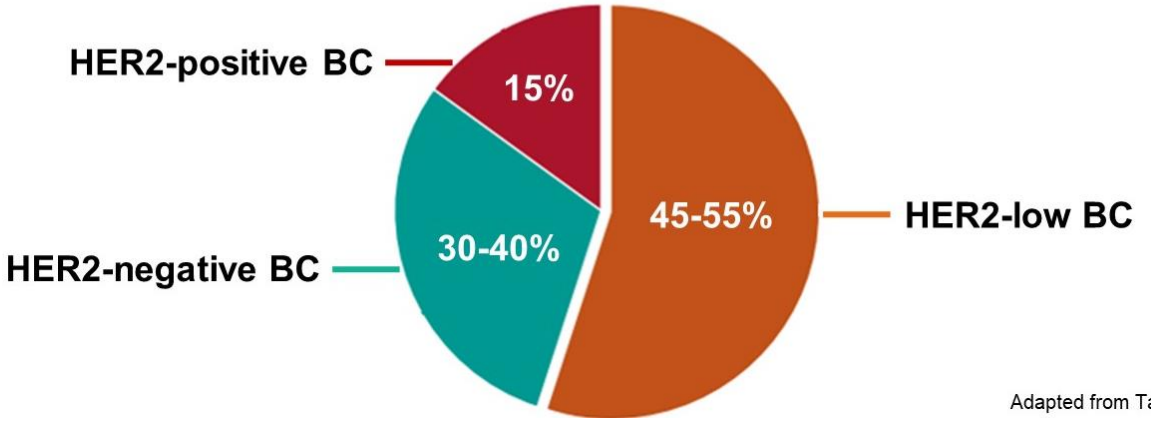
Stratification factors
- Centrally assessed HER2 status[d] (IHC 1+ vs IHC 2+/ISH−)
- 1 versus 2 prior lines of chemotherapy
- HR+ (with vs without prior treatment with CDK4/6 inhibitor) versus HR−



N Engl J Med. 2022 PMID: 35665782

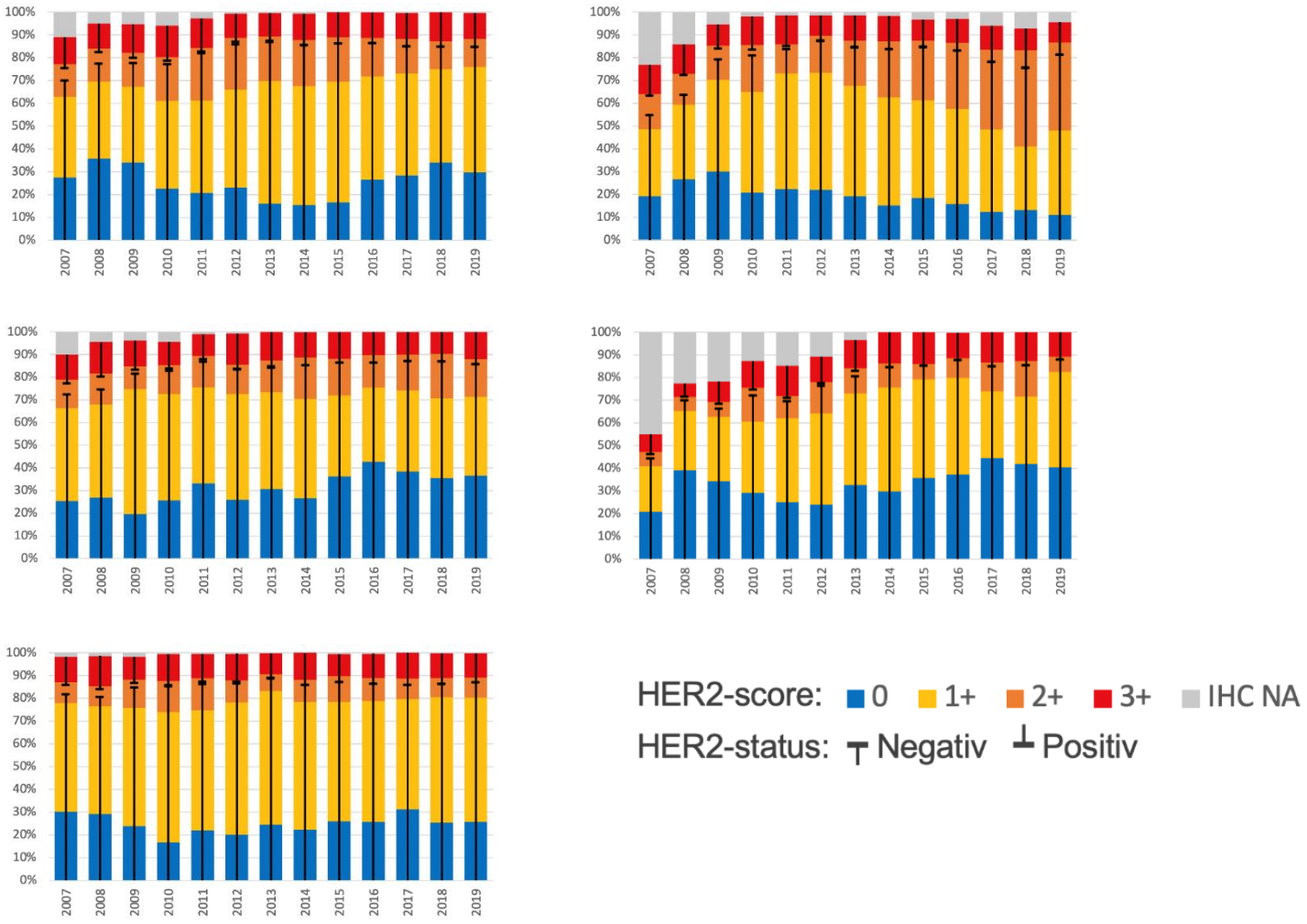
Algoritm for defining HER2 low BC





Adapted from Tarantino et al. J Clin Oncol. 2020 38(17)

HER2-low: Variation over time



DPAS årsmøde 2022 v. PhD studerende cand. med. Kåre Nielsen

With this new class of anti-cancer agents, we must also rethink novel, more accurate and sensitive ways of assessing Her2 status

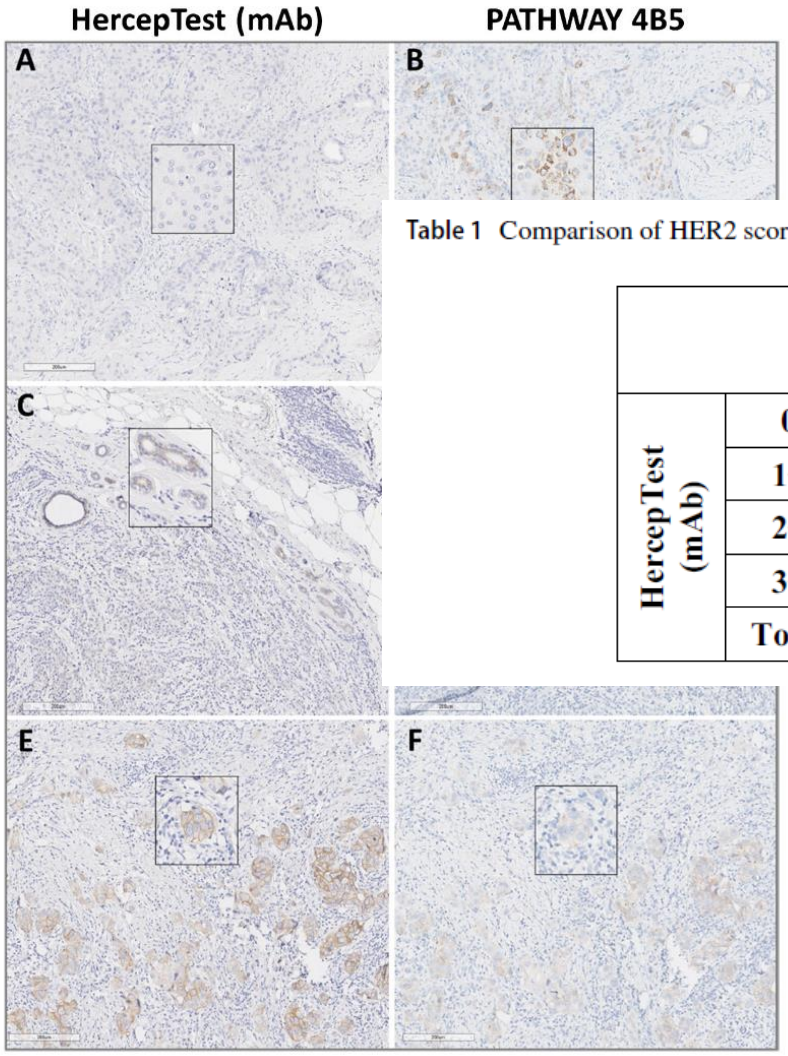
HER2 assay – sensitivity?
When IHC - which assay to use
PD-L1 challenge revisited

Virchows Archiv
https://doi.org/10.1007/s00428-022-03378-5

ORIGINAL ARTICLE

Comparison of HercepTest™ mAb pharmDx (Dako Omnis, GE001) with Ventana PATHWAY anti-HER-2/neu (4B5) in breast cancer: correlation with *HER2* amplification and HER2 low status

Josef Rüschoff[1] · Michael Friedrich[1] · Iris Nagelmeier[2] · Matthias Kirchner[2] · Lena M. Andresen[3] · Karin Salomon[3] · Bryce Portier[4] · Simone T. Sredni[4] · Hans Ulrich Schildhaus[1,2] · Bharat Jasani[1] · Marius Grzelinski[1] · Giuseppe Viale[5]



Results

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

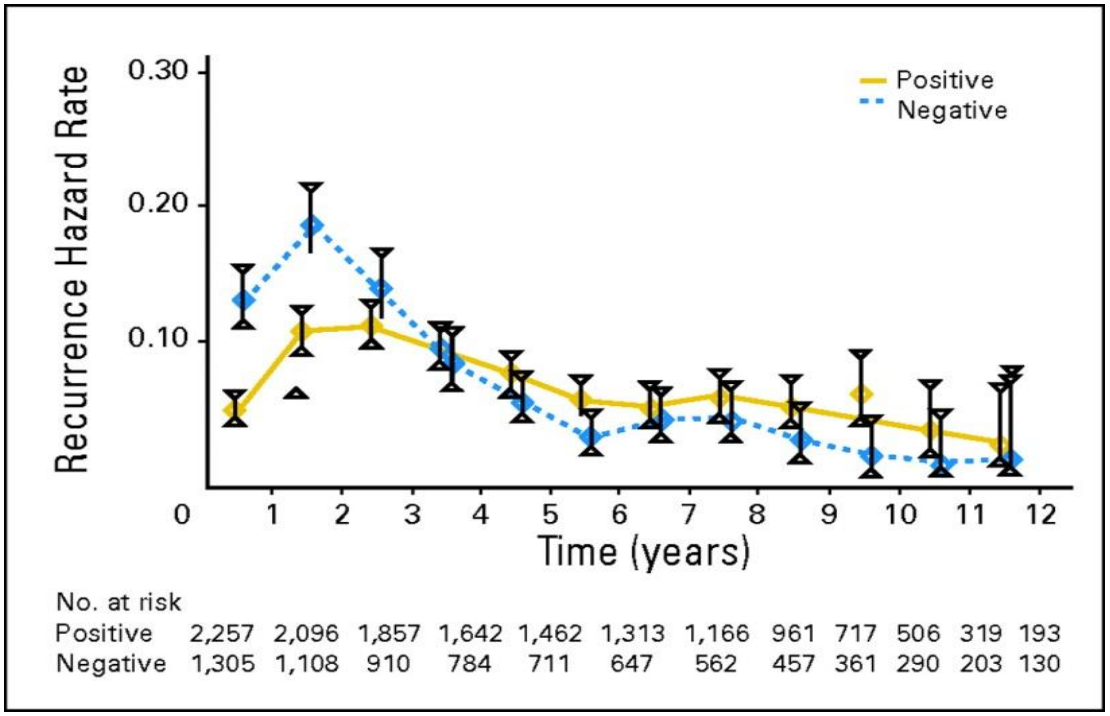
		PATHWAY 4B5				
		0	**1+**	**2+**	**3+**	**Total**
HercepTest (mAb)	**0**	35	0	0	0	**35**
	1+	17	8	0	0	**25**
	2+	4	12	13	1	**30**
	3+	0	0	2	27	**29**
	Total	**56**	**20**	**15**	**28**	**119**

The amount of HER2 Low cases increased markedly (Herceptest GE001 vs Pathway 4B5)

Virchows Archiv
https://doi.org/10.1007/s00428-022-03378-5

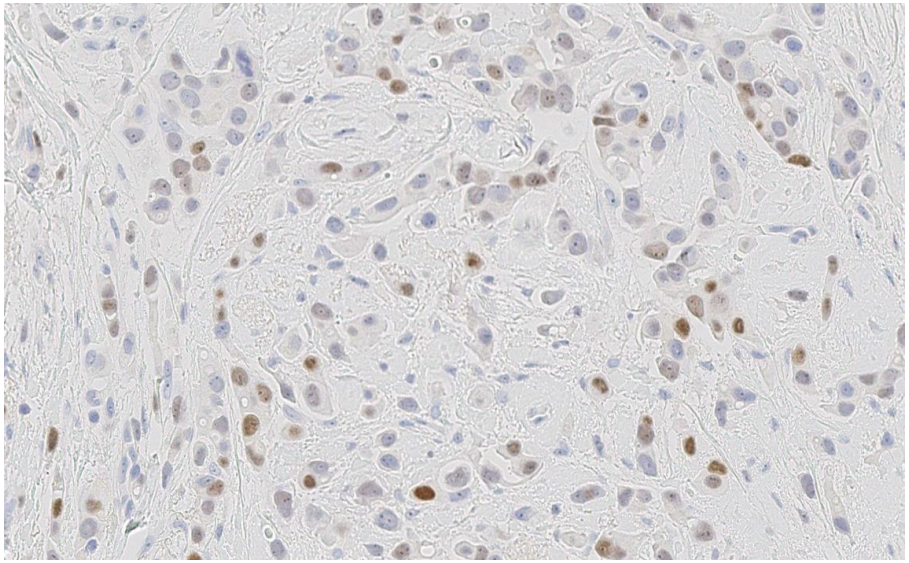
The Estrogen receptor as a prognostic/predictive marker

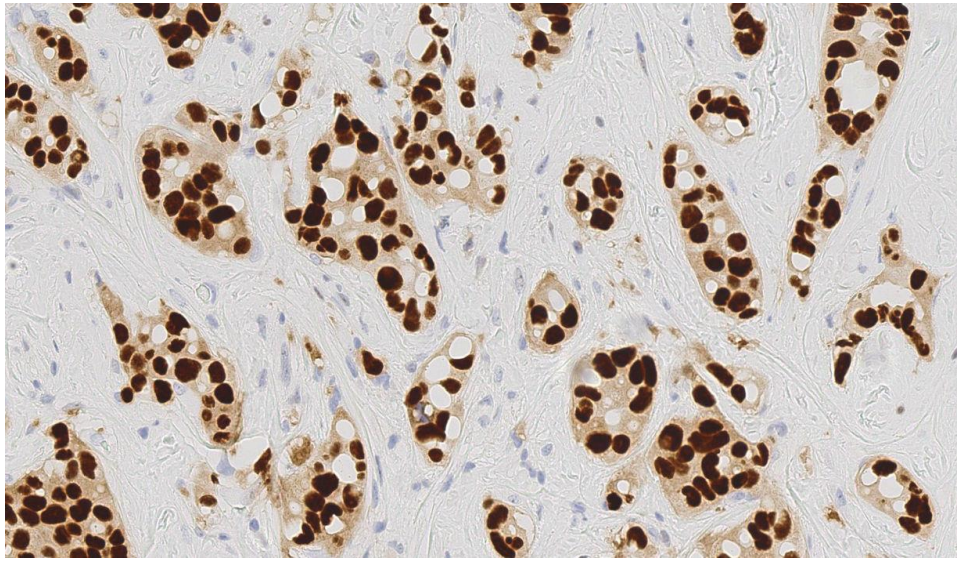
Risk of recurrence pr. year
N = 3,562 patients



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

2020 – ASCO CAP Update
Hormone receptors

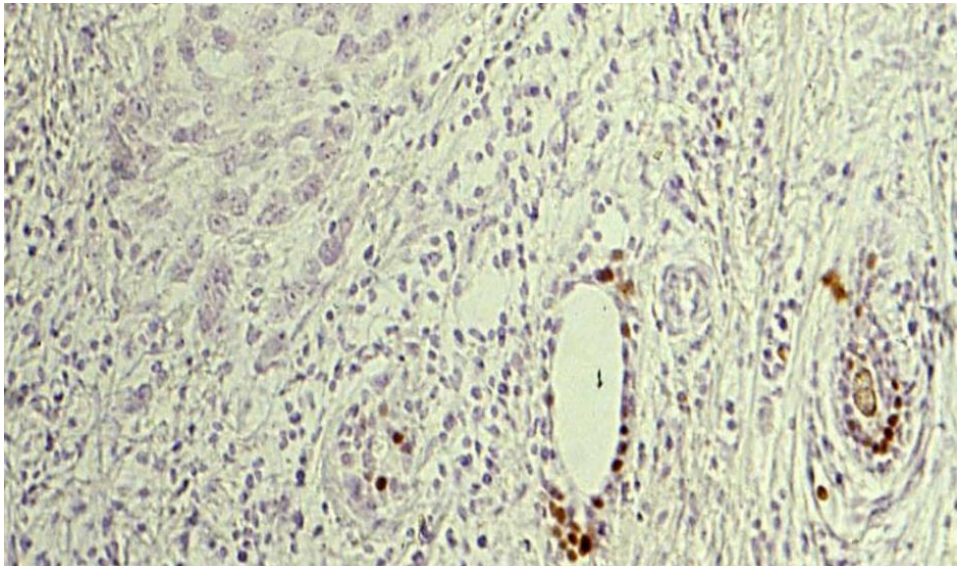




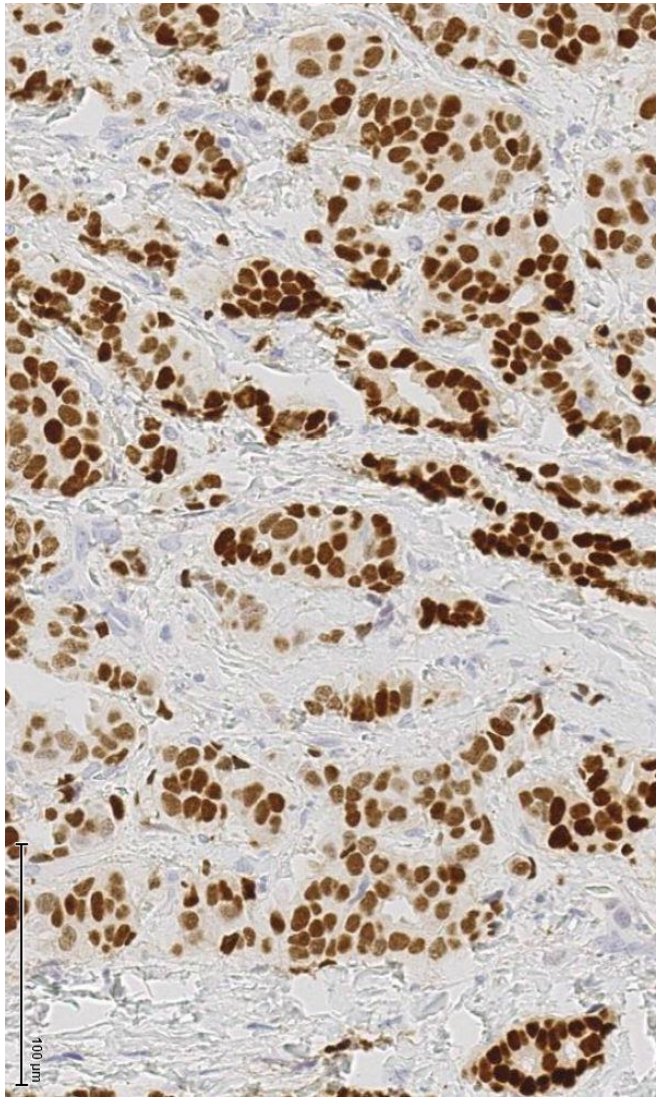
ER positive 86% of breast carcinomas (DK)

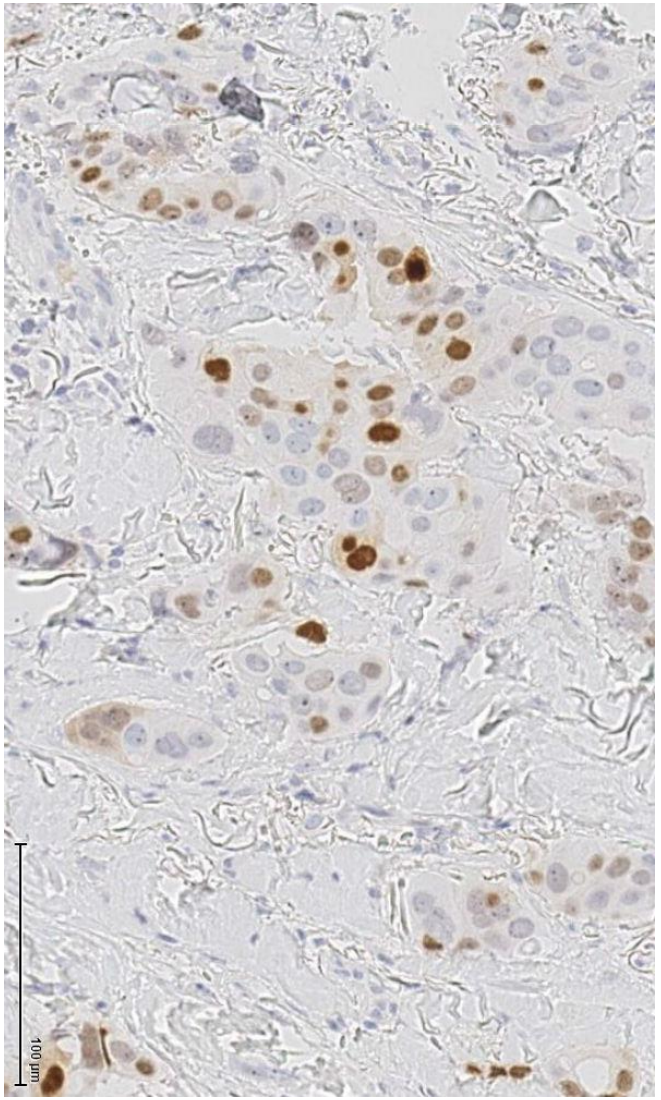
Cut off ≥ 1%
A sample is reported negative for ER or PgR if < 1% or 0% of tumor cell nuclei are immunoreactive.

Limited data on the overall benefit of endocrine therapies for patients with low level (1-10%) ER expression.



Interpretation of PgR

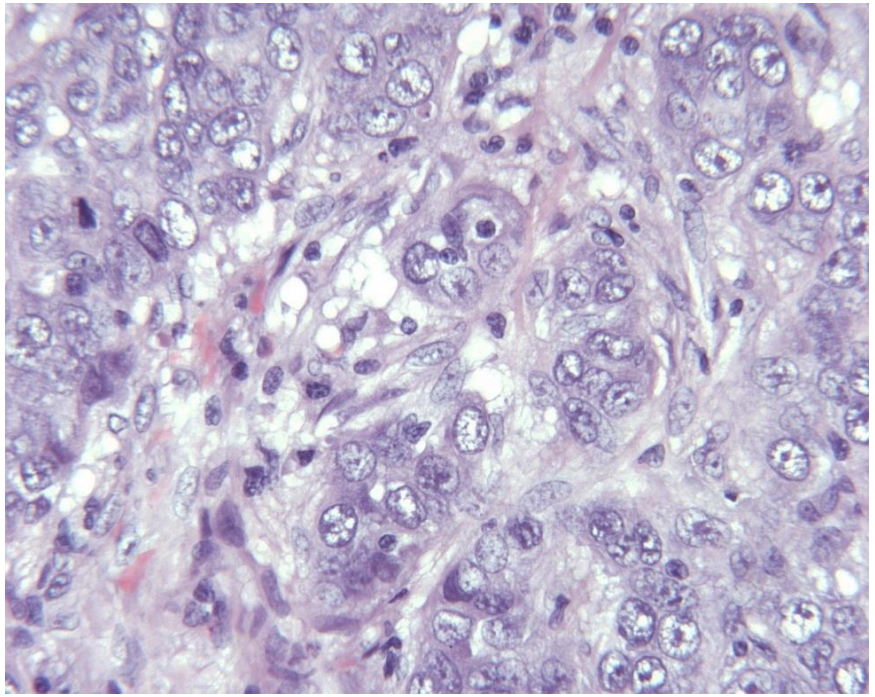




Heterogeneous expression

TNBC : 8-10% of primary breast cancers

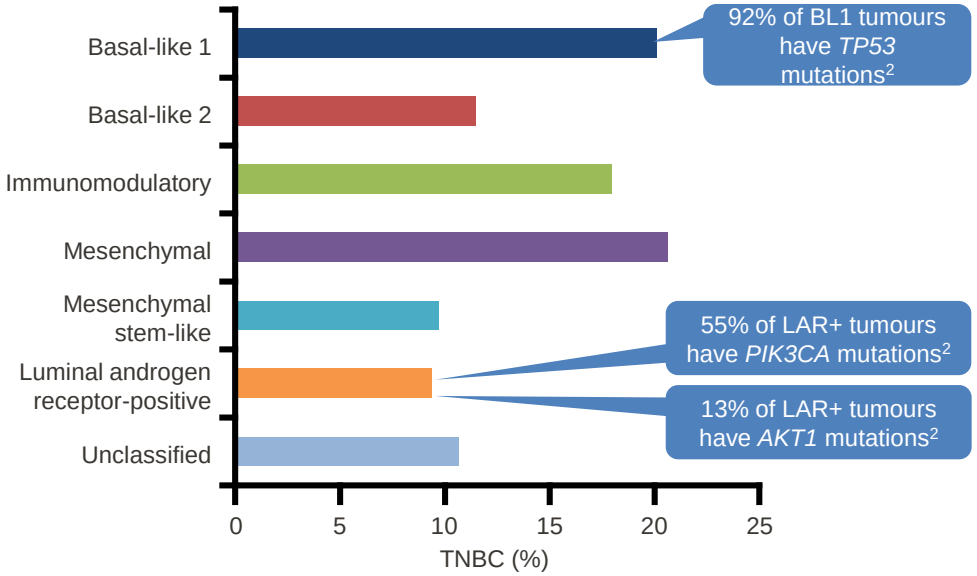
- ER, PR and HER2 negative
- Heterogeneous group of tumours
- High grade
- Younger age at diagnosis
- Poor prognosis
- Risk of *gBRCA* mutation



Heterogeneity of TNBC

- TNBC is a combination of many disease entities that have been grouped together for ease of clinical categorization.
- But studies reveal a high level of heterogeneity[1–3]
  - High levels of genetic instability versus other BC subtypes
  - Complex patterns of copy number alterations and structural rearrangements
- *PIK3CA/AKT1/PTEN* alterations are seen in ~24%[4]
- *BRCA1/2* mutations are seen in ~20%[5]

Six unique molecular subtypes of TNBC have been identified[6]

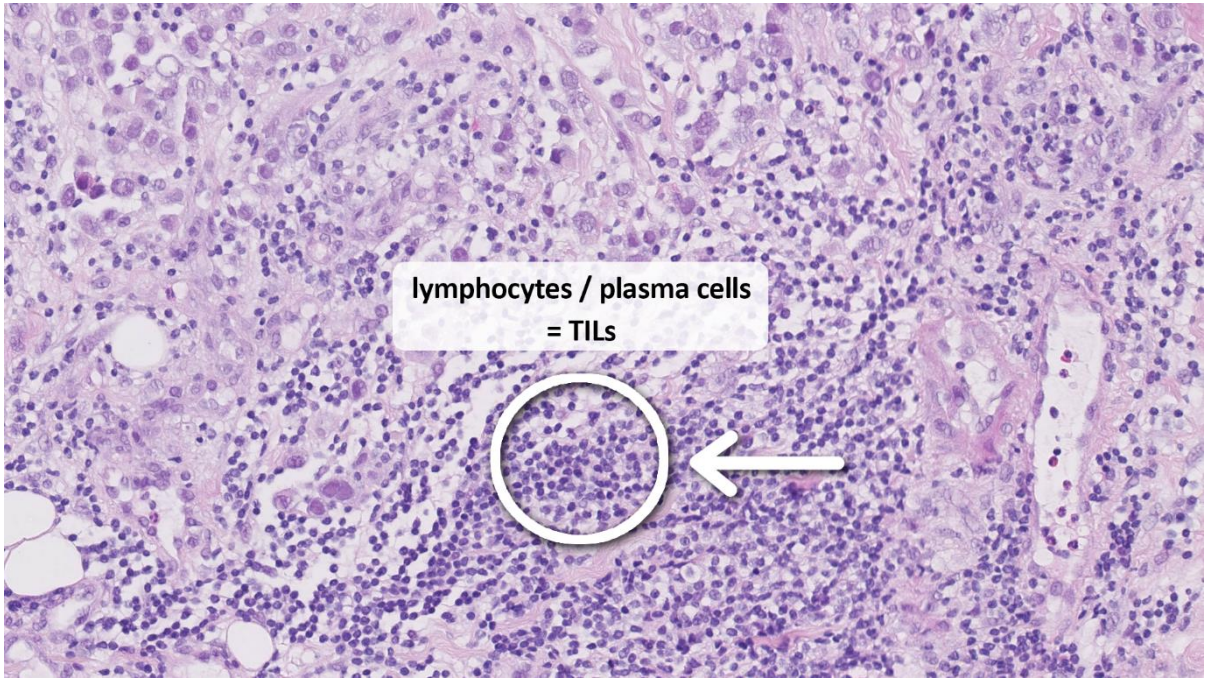


1. Lehmann, et al. J Clin Investig 2011; 2. Bareche, et al. Ann Oncol 2018

3. TCGA, Nature 2012; 4. Schmid, et al. ASCO 2015
5. Gonzalez-Angulo, et al. Clin Cancer Res 2011; 6. Abramson et al. Cancer 2015

Figure reproduced from Abramson 2015[6]

Tumor infiltrating lymphocytes and TNBC



TNBC is considered to be the most immunogenic breast cancer subtype, with a higher median number of tumor-infiltrating lymphocytes (TILs), PD-L1 expression, both markers associated with tumor microenvironment (TME) immune activity.

Level 1B evidence / prognostic marker

Loi, S., et al., *Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers.* J Clin Oncol, 2019. **37**(7): p. 559-569.

Triple-Negative Breast Cancer Histological Subtypes with a Favourable Prognosis

The majority of TNBC are invasive ductal carcinomas (IDC) – Figure 1
Rare special histological subtypes are low proliferative tumours with good prognosis allthough being triple negative (Figure 2 and 3).
Cserni G et al. Cancers 2021, PMID: 34830849

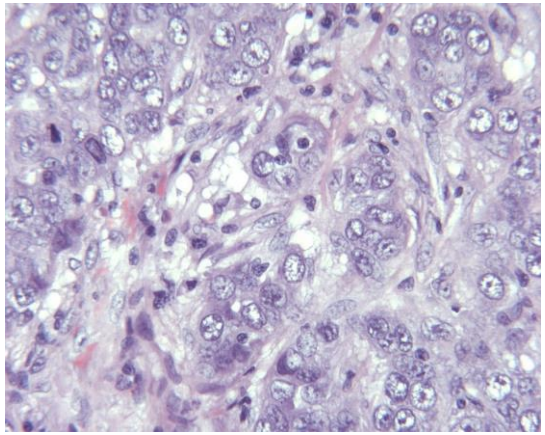


Figure 1
High grade IDC

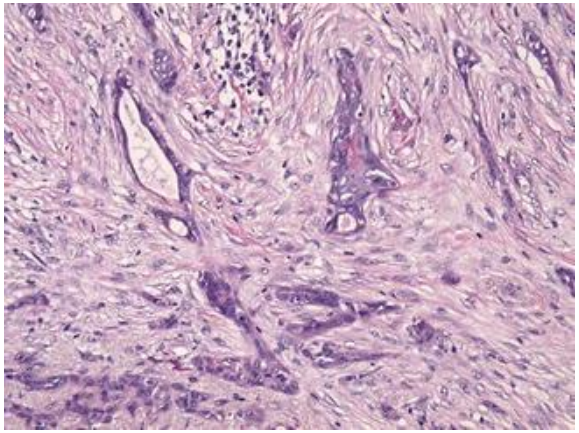


Figure 2
Low grade adenosquamous carcinoma (subtype of metaplastic carcinoma)
luminal (CK7, CK8) and basal (CK5, CK14) CKs and squamous (myoepithelial) markers p63 and p40.

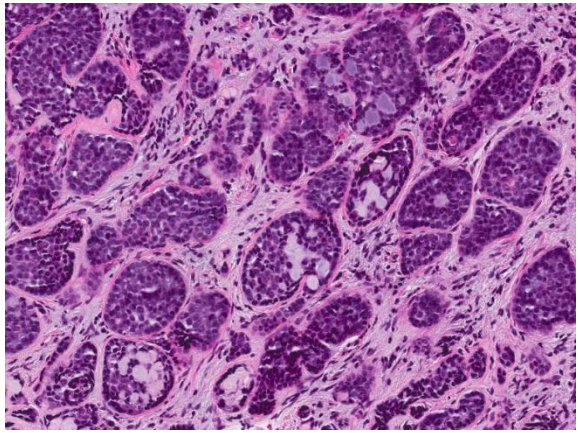
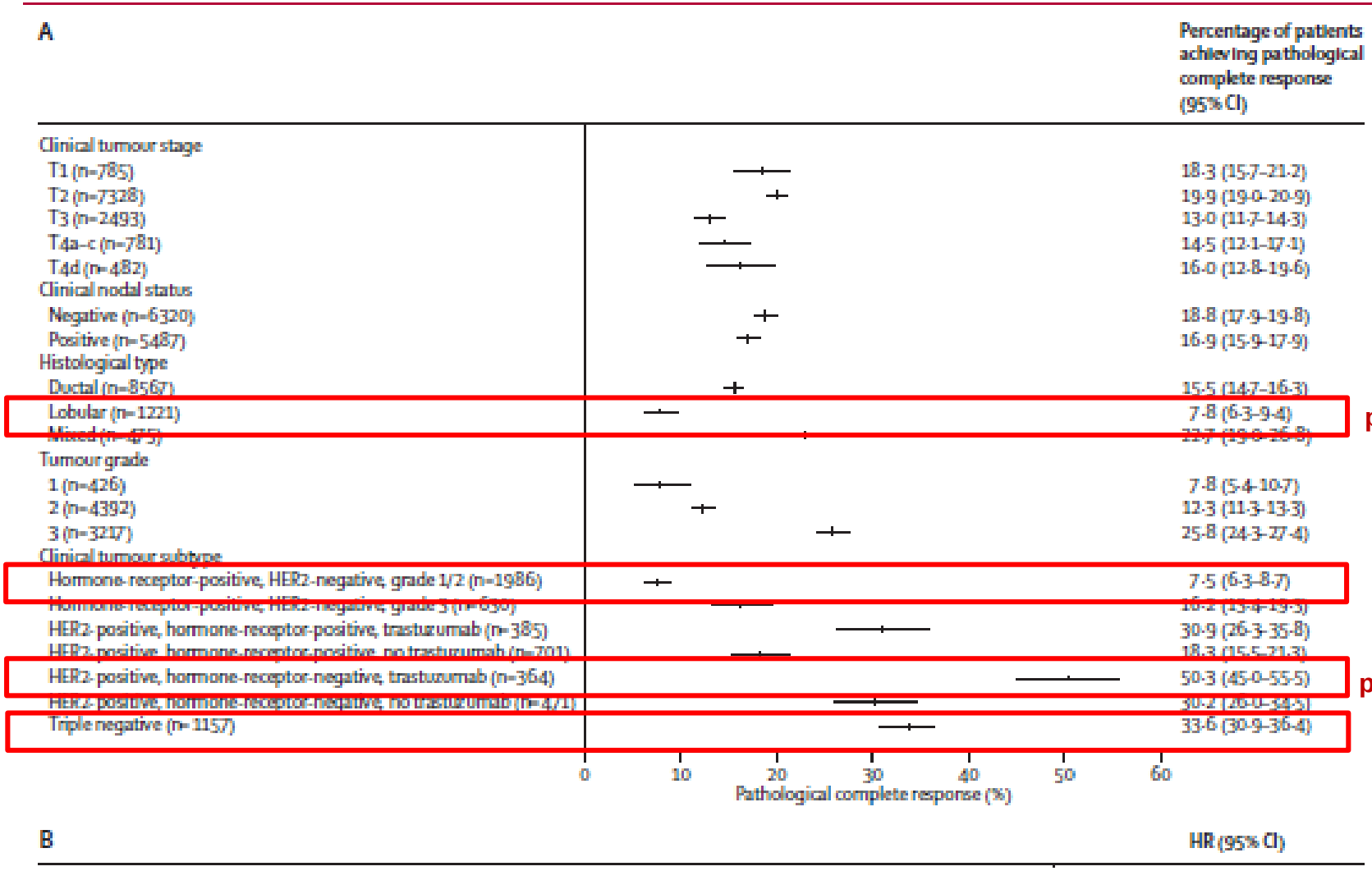


Figure 3
Adenoid cystic carcinoma of the breast. The cells of the epithelial component are positive for CK7, CK5/6, CK 8/18 and CD117. The myoepithelial /abluminal cells express p63, smooth muscle actin and basal CKs: CK5/6, CK14, CK17.

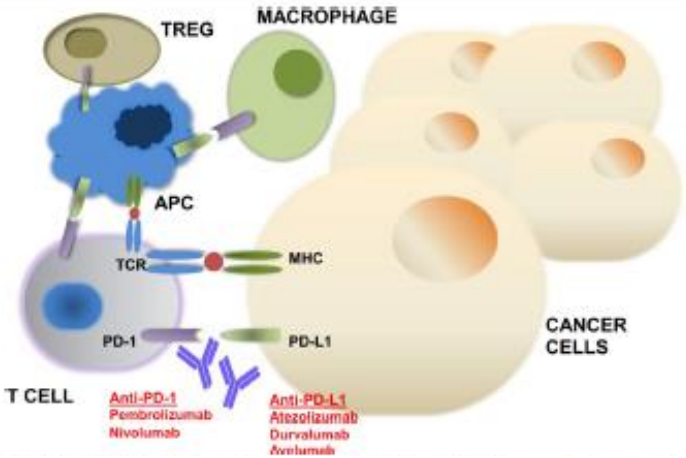
Tumor characteristics and association with pCR
Neoadjuvant chemotherapy (NACT)



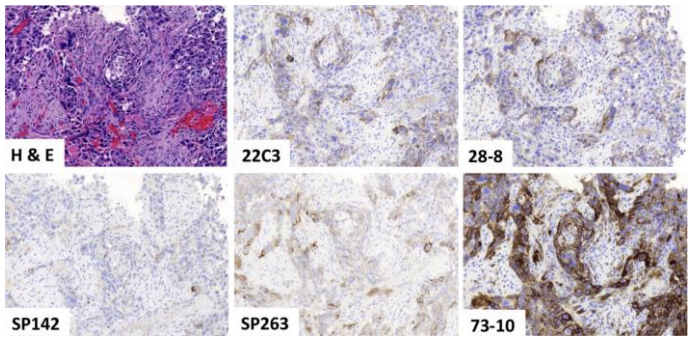
Cortazar et al. Lancet 2014; 384: 164-72

PD-L1
in TNBC

Mechanism of action of PD-1 and PD-L1 inhibitors



PD-L1 is expressed on lymphocytes, macrophages, fibroblasts, tumour cells.



Binding of PD-1 to its ligand PD-L1 results in suppression of proliferation and immune response of T cells. Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade antigen-specific T-cell immunologic responses.
Antibody blockade of PD-1 or PD-L1 reverses the process and enhances antitumor immune activity

Gong et al. Journal for ImmunoTherapy of Cancer (2018) 6:8

PD-L1 scoring system

Which scoring system should be used for PD-L1 staining?

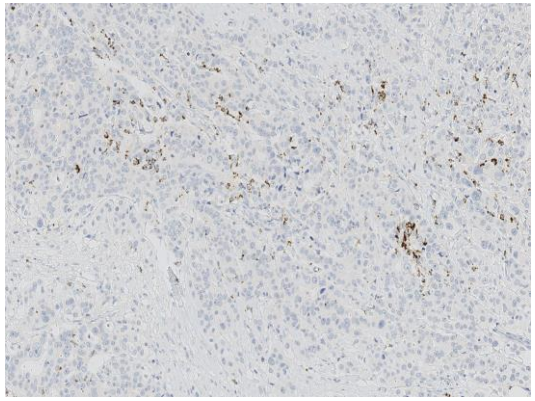
	IVD diagnostic antibodies used in clinical trials	
Drug	Pembro-lizumab **(MSD)**	Atezo-lizumab **(Roche)**
AB clone	22C3 Dako	SP-142 Ventana
Score	CPS	IC_A
cell type	Tumor Immune	Immune
Breast cancer trial	KN-012 KN-522	**Impassion -130**

IC_A score: percentage of tumor area covered by PD-L1 positive immune cells (designed for Atezolizumab)

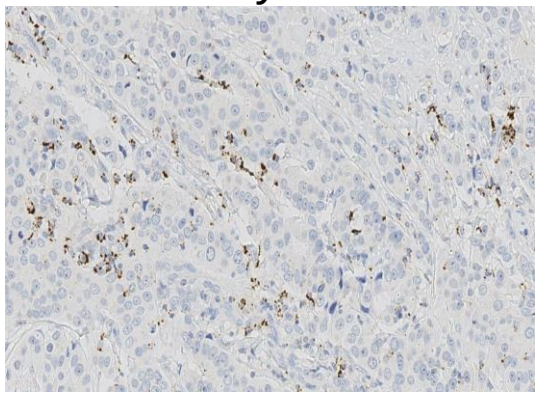
CPS score: positive tumor or immune cells as percentage of all tumor cells (designed for Pembrolizumab)

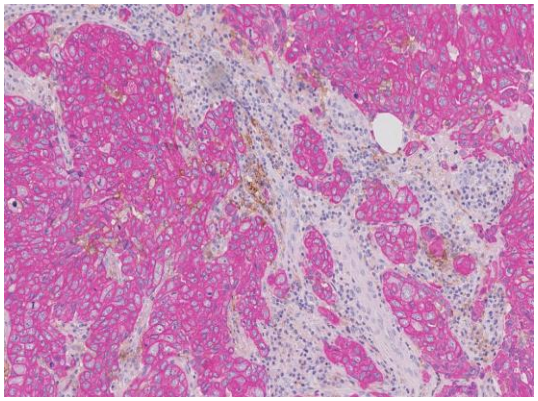
Keynote-355 Impassion130

PD-L1 immunohistochemistry

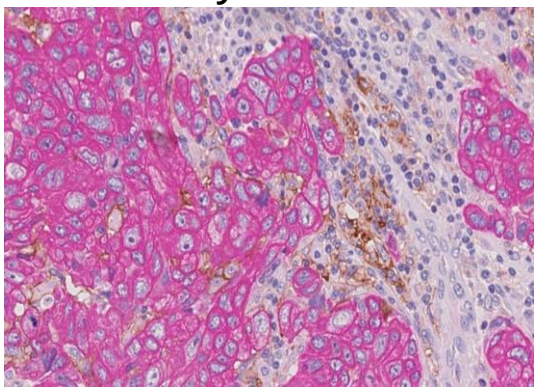


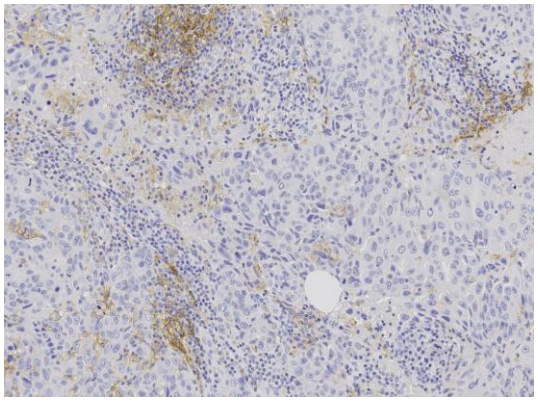
Assay SP142



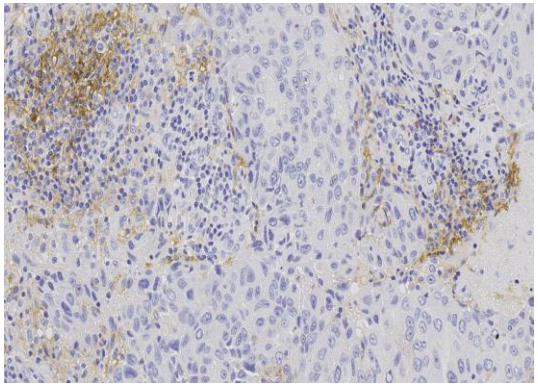


Assay 22C3+CK8



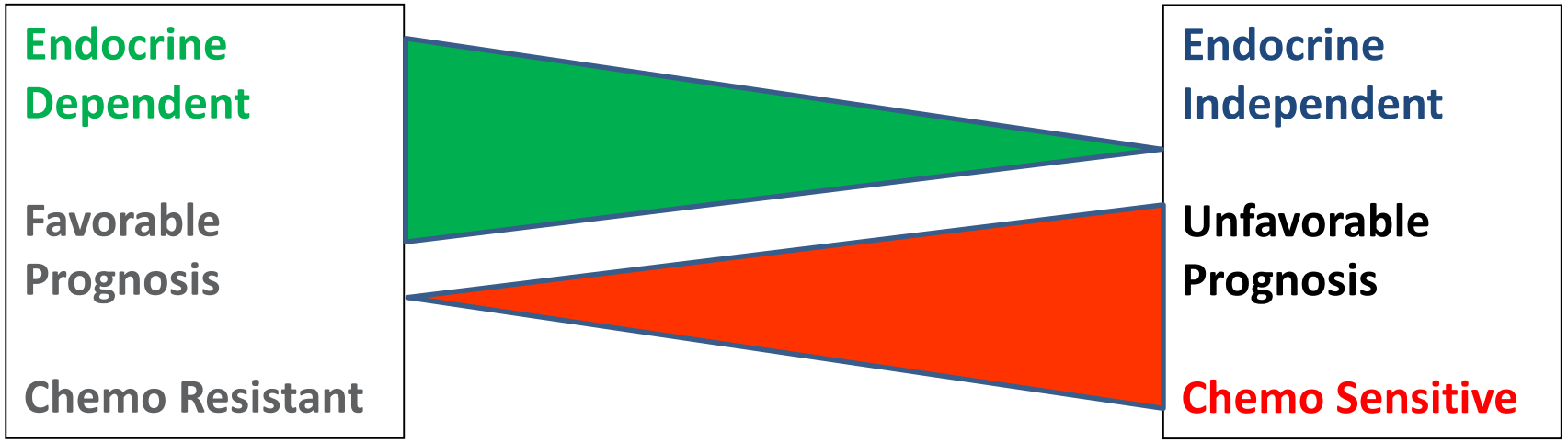


Assay 22C3



courtesy of Ulriche Thiim Pedersen

Breast cancer – Molecular intrinsic subtypes prognostic information



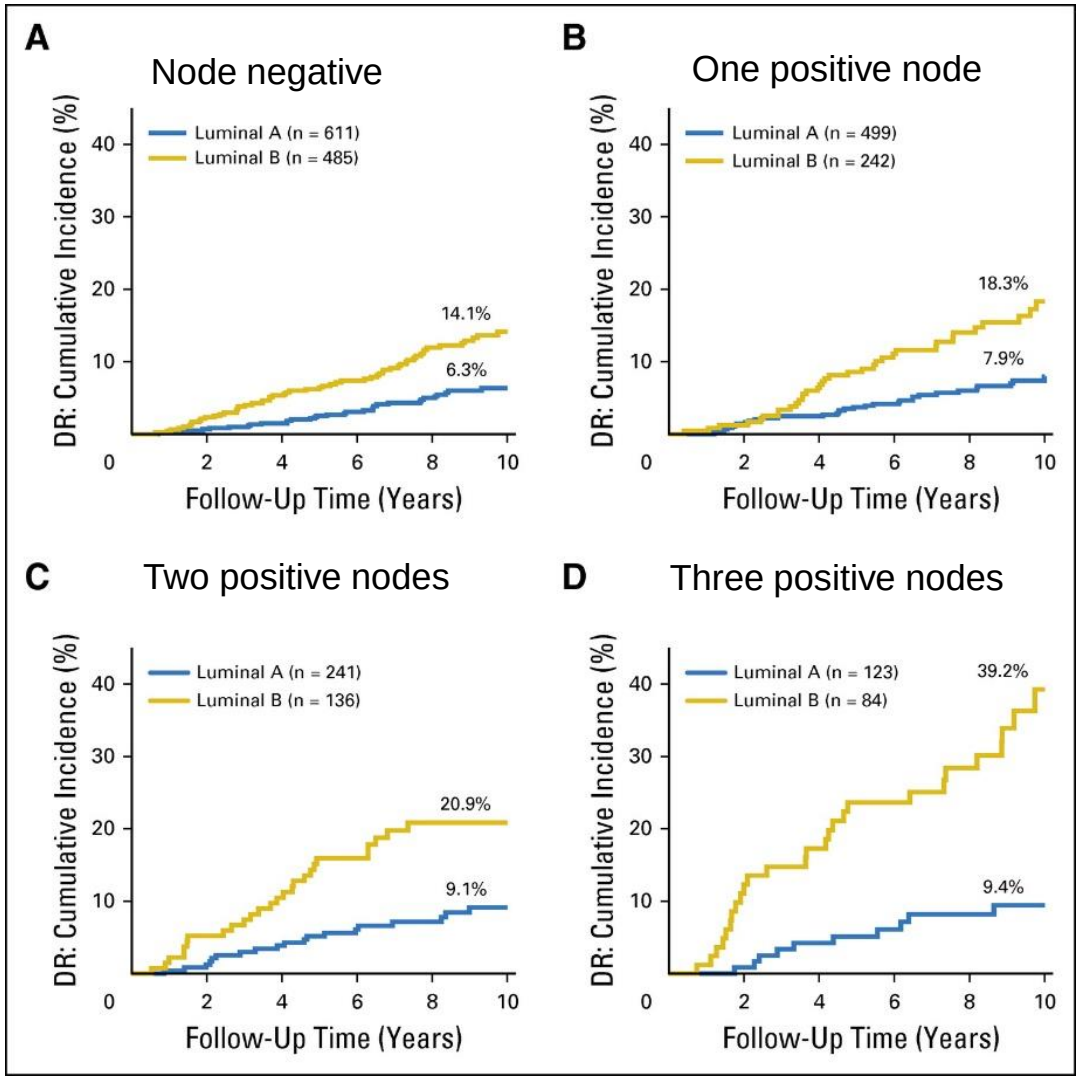
Lum A

Lum B

HER2-enriched

Basal-like

De-escalation of treatment
More patients can be spared chemotherapy



Luminal A; and Luminal B

JCO 2018 Laenkholm et al.

PAM50 implemented in the Danish guidelines

Immunohistochemical surrogate markers for the molecular intrinsic subtypes

- Limitations
  - No uniform cut off value for Ki67
  - Lack of analytical validity - reproducebility
  - Lack of correlation between molecular subtypes and surrogate IHC subtypes

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 Dowsett, Torsten O. Nielsen, Roger A'Hern, John Bartlett, R. Charles Coombes, Jack Cuzick, Matthew Ellis, N. Lynn Henry, Judith C. Hugh, Tracy Lively, Lisa McShane, Soon Paik, Frederique Penault-Llorca, Ljudmila Prudkin, Meredith Regan, Janine Salter, Christos Sotiriou, Ian E. Smith, Giuseppe Viale, Jo Anne Zujewski, Daniel F. Hayes

OXFORD

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

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Commentary

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

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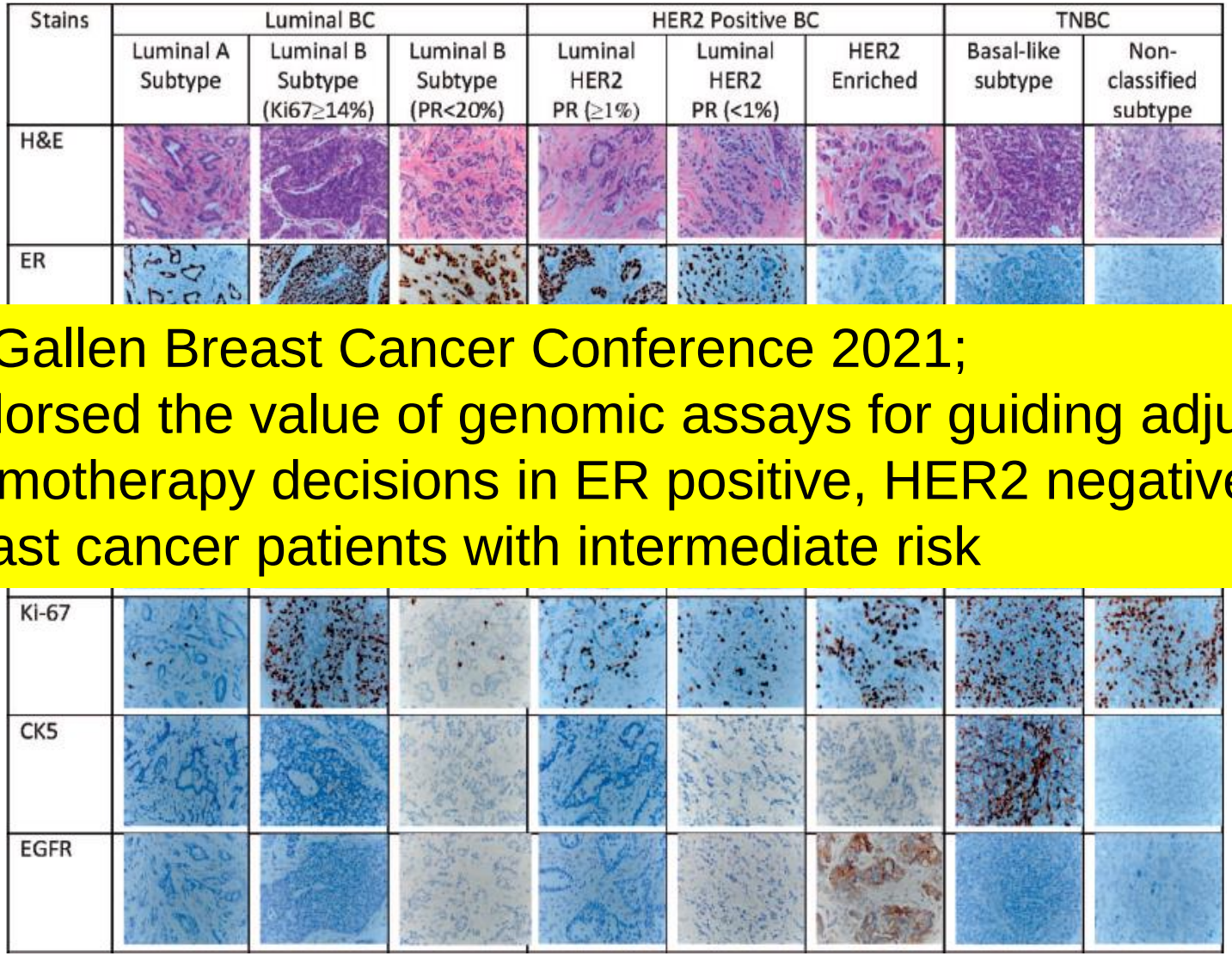
Immunohistochemical surrogate markers for the molecular intrinsic subtypes

Arch Pathol Lab Med—Vol 140, August 2016

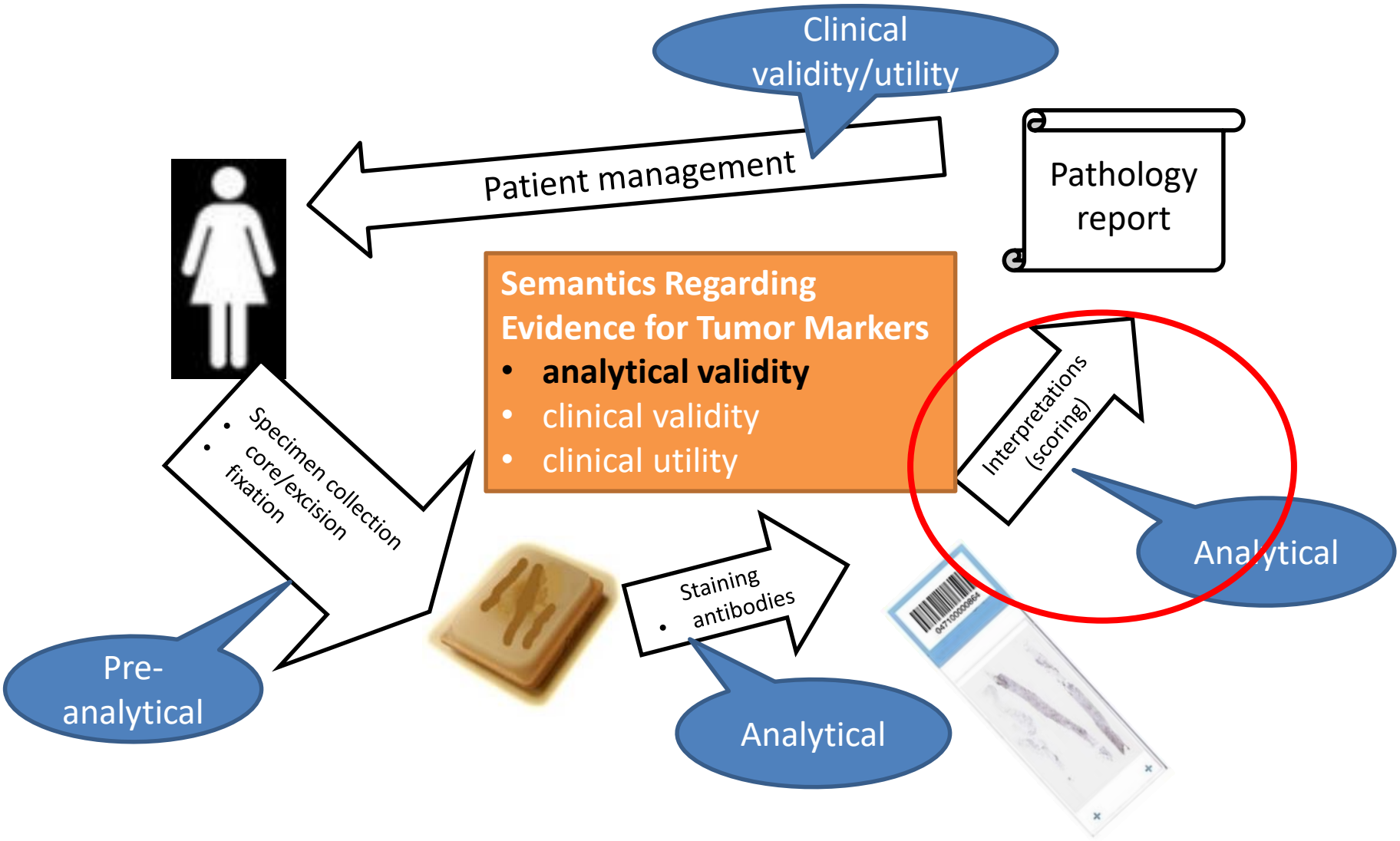


St. Gallen Breast Cancer Conference 2021;
Endorsed the value of genomic assays for guiding adjuvant chemotherapy decisions in ER positive, HER2 negative breast cancer patients with intermediate risk

In conclusion
IHC for diagnostic use in 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
  - Prognostic and predictive factors
    - Assay preference and treatment
    - Tumor heterogeneity
- Intrinsic molecular subtype / gene expression profile
  - Identification of patients who can be spared chemotherapy
- Focus on analytical validity
- External quality assurance program

Evidence for Tumor Markers




Thank you!